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PUBLIÉES PAR GIUSEPPE MORUZZI
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TOME XCVIII - Fasc. 1
Paru le 20 Février 1960

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THE RIGHT SUBCLAVIAN BODY[1]

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INTRODUCTION

Glomus tissue similar in histological structure to the carotid body has been described in mammals in the vicinity of the origin of the right subclavian artery in the angle between this vessel and the right common carotid (15, 16, 17, 20, 12). A similar formation has also been found in the concavity of the right subclavian artery itself (15, 18, 21).

It is well known that the carotid body has a chemoreceptor function and a similar function has been demonstrated in connection with other epithelioid aggregates situated in the concavity of the aortic arch (11, see pp. 131-142) and on the ventral surface of the aorta near the origin of the left subclavian artery (7), the so-called aortic bodies. The glomus tissue situated near the root of the right subclavian artery would also appear to have a chemoreceptor function, but no clear experimental evidence of this fact has so far been presented.

This paper presents an electroneurographic study of the activity of the right subclavian body, demonstrating that it has, in fact, a chemoreceptor function.

METHODS

Adult cats were anaesthetized with pentobarbitone (60 mg/kg) injected intraperitoneally. The right aortic nerve was identified in the vicinity of the superior laryngeal nerve and cut centrally. Few-fibre preparations were made from it. They were individually laid on saline wick electrodes in connection with resistance-capacity amplifiers and tested for chemoreceptor impulse activity under spontaneous ventilation of the animal through valves in 5% O_2 and 100% O_2. A loudspeaker and a long persistance C.R.O. were used as monitors; records were taken by means of a Cossor oscillographic camera, Model 1049 MK IIIA. Occasionally, from the right aortic nerve were obtained preparations containing chemoreceptor fibres only.

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[1] A preliminary note was published in *J. Physiol.* London (1).

[2] On leave of absence from the Physiology Department of the University of Parma, Italy.

The nerve to baroreceptor area 1 (9; 11, see pp. 5-6) was identified in the angle between the right common carotid and subclavian arteries; the nerve to baroreceptor area 1′ (3) was identified in the concavity of this last artery (Fig. 1). Both nerves were cut centrally and the residual chemoreceptor activity in the right aortic nerve preparation was tested after each section; both nerves were then individually tested for chemoreceptor activity.

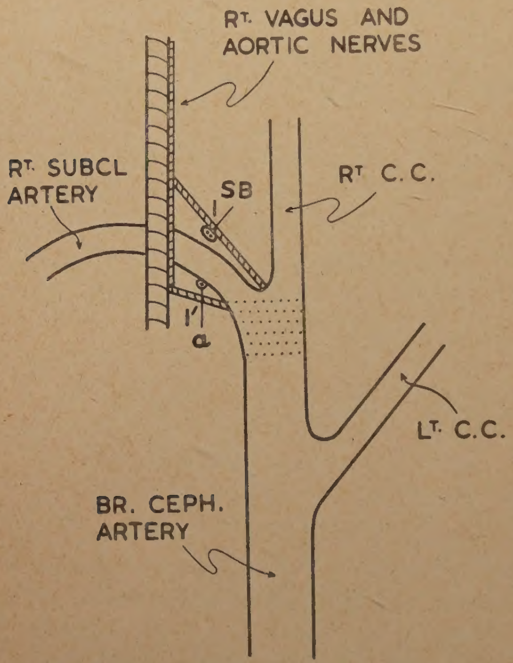

Fig. 1. – *Diagram showing the site of the right subclavian body in the angle of the bifurcation of the brachiocephalic trunk into right common carotid and right subclavian arteries.*

S. B.: right subclavian body; 1: nerve to baroreceptor area 1; a: additional right subclavian body; 1′: nerve to baroreceptor area 1′; Rt. subcl. art.: right subclavian artery; Rt. C. C.: right common carotid; Br-ceph. art.: brachio-cephalic artery or trunk.

To expose the nerve to baroreceptor area 1′, the chest was opened and the superior vena cava retracted; the animal was kept on positive pressure artificial respiration.

Histological controls were performed, the region of the bifurcation of the brachio-cephalic trunk being removed and fixed in formol-saline. Serial paraffin sections were cut at 10 μ thickness and stained with eosin-haematoxylin.

RESULTS

1. *Effect of the section of the nerve to baroreceptor area 1 and of the nerve to baroreceptor area 1′ on the right aortic nerve chemoreceptor activity.* — A typical record of the chemoreceptor activity in the

right aortic nerve is shown in Fig. 2. The record has been taken from a preparation containing chemoreceptor fibres only. The impulse activity, which was slight while the animal was breathing air spontaneously, increased a little as soon as the animal breathed through valves which presumably slightly restricted its ventilation. Severe anoxia (ventilation in 5% O_2) or asphyxia caused a considerable increase in the impulse activity, that subsided under subsequent ventilation in 100% O_2.

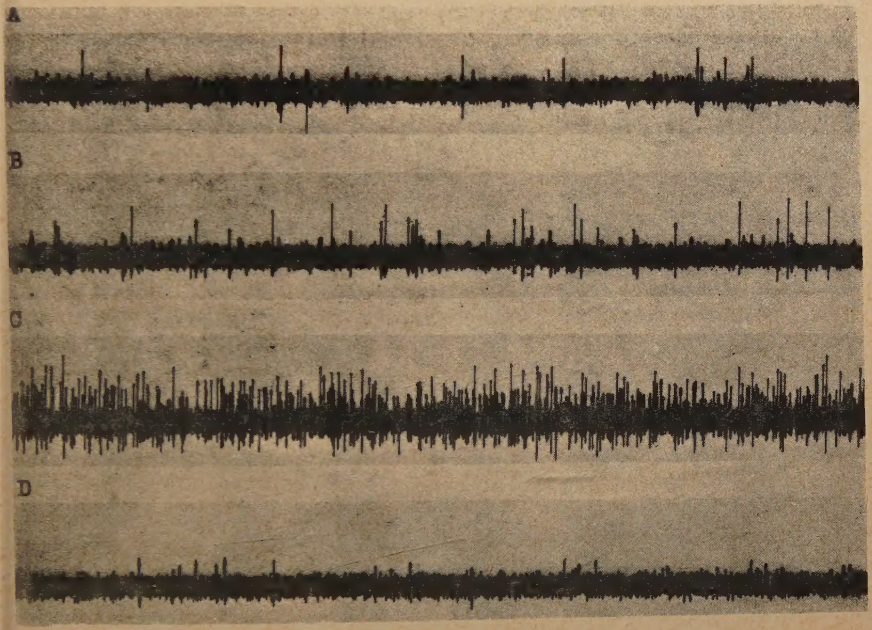

Fig. 2. — *Right aortic nerve chemoreceptor activity.*

Cat. Sodium pentobarbitone anaesthesia (60 mg/kg intraperitoneal). Right aortic nerve cut at its origin from the superior laryngeal nerve. Oscillographic records from a right aortic nerve preparation containing only chemoreceptor fibres. *A*: respiration in air; *B*: resp. in air through valves; *C*: resp. in 5% O_2; *D*: resp. in 100% O_2.

The majority of the chemoreceptor activity in the right aortic nerve, shown in Fig. 2, originates from the aortic bodies situated in the aortic concavity (Penitschka's body; 22) and joins the main vagus trunk at the level of the right subclavian artery (2). The subclavian body described by Muratori and by Nonidez is situated in the angle of the bifurcation of the brachio-cephalic trunk into right common carotid and right subclavian arteries. It seemed likely that its chemoreceptor fibres would course with those of the corresponding baroreceptor area before finally joining the aortic nerve.

The presence, in the concavity of the subclavian artery, of a branch of the aortic nerve which contains the baroreceptor supply to the distal end of the brachio-cephalic trunk (nerve to area 1'; 3) could provide a pathway for chemoreceptor fibres taking origin in the glomus tissue situated below the concavity of the subclavian artery at the site of the body described by Boyd.

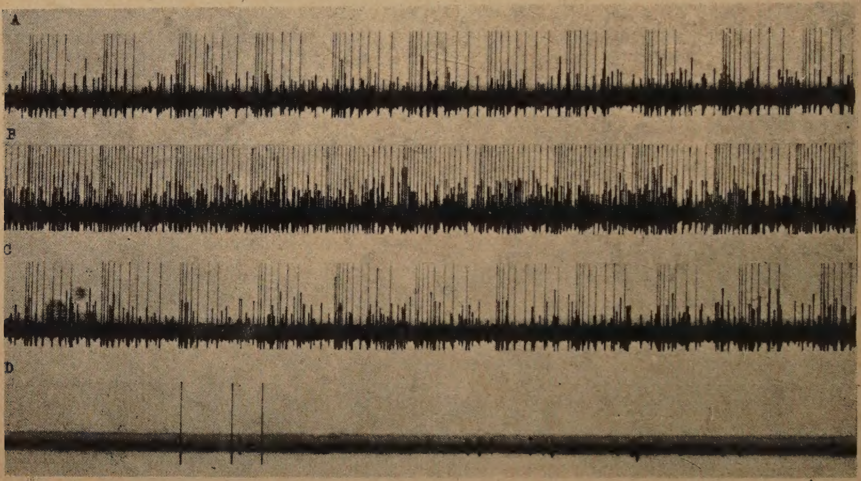

Fig. 3. – *Impulse activity disappears in a few fibre preparation of the right aortic nerve on section of nerve to area 1.*

Cat. Sodium pentobarbitone anaesthesia (60 mg/kg intraperitoneal). Right aortic nerve cut at its origin from the right superior larygeal nerve and nerve to area 1 isolated in the right subclavian-carotid angle. Oscillographic records from a few-fibre preparation of the right aortic nerve. Large spikes, baroreceptor; small spikes, chemoreceptor. *A*: respiration in air; *B*: resp. in 5% O_2; *C*: resp. in air; *D*: 5 min after section of nerve to area 1.

In the preparations of the aortic nerve showing chemoreceptor activity arising only from the subclavian body, section of the nerve to baroreceptor area 1 or of the nerve to area 1' caused immediate disappearance of the impulse activity, depending on the area of distribution of the nerve thus cut. An example of this is shown in Fig. 3. In this case the section of the nerve to area 1 caused complete disappearance of the chemoreceptor activity.

2. *Chemoreceptor activity in the nerves to baroreceptor areas 1 and 1′.* — Chemoreceptor activity has been found in few-fibre preparations made from the nerve to baroreceptor area 1, but detection is difficult because of the high density of large baroreceptor action potentials which tend to mask the smaller chemoreceptor action potentials. In Fig. 4 the chemoreceptor activity may be seen in the diastolic pauses of baroreceptor activity. This record shows that the chemoreceptor impulse activity is markedly increased by anoxia and is greatly reduced by ventilation in 100% O_2. The change in activity was very obvious in the sound produced by the loudspeaker monitor.

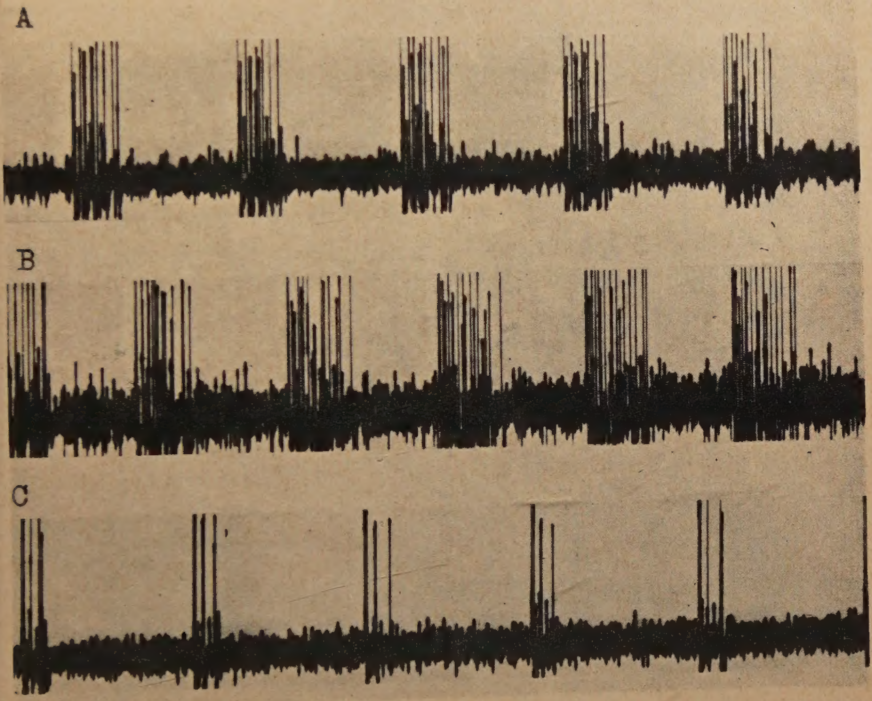

Fig. 4. — *Baro- and chemoreceptor activity of area 1 nerve.*

Cat. Sodium pentobarbitone anaesthesia (60 mg/kg intraperitoneal). Nerve to area 1 found in the angle between right common carotid and right subclavian arteries. Oscillographic records from a few-fibre preparation of nerve to area 1. *A* : respiration in air; *B* : resp. in 5% O_2; *C* : resp. in 100% O_2.

Haemorrhage is a powerful chemoreceptor stimulant (4, 14, 7). Fig. 5 shows the effect of haemorrhage on the chemoreceptor activity in the nerve to area 1. A gradually increasing impulse activity was found, that became marked after a haemorrhage of 50 ml of blood had occurred. Replacement of the blood stopped such activity.

Lobeline (0.3 mg/kg i. v.) (Heymans, Bouckaert and Dautrebande, 10) was also effective in stimulating chemoreceptor impulse activity from these fibres preparations.

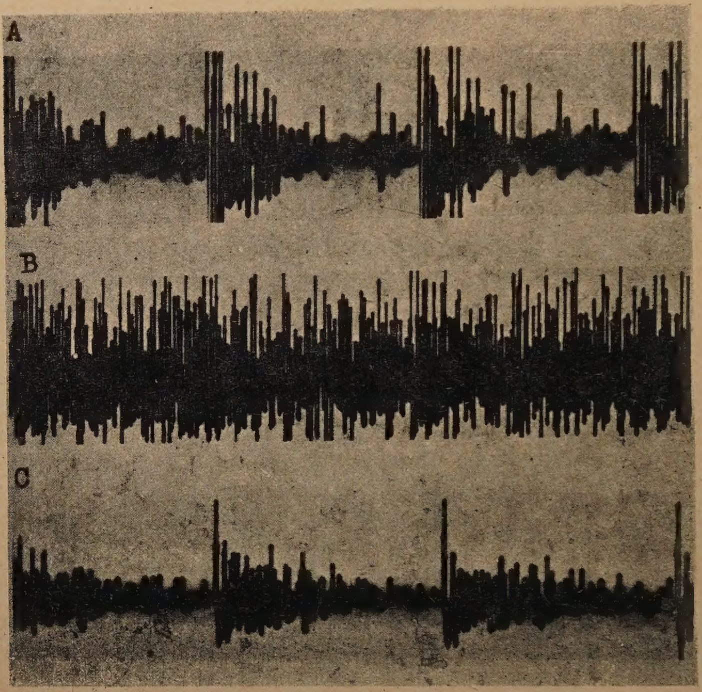

Fig. 5. – *Stimulation of the right subclavian body by haemorrhage.*

Cat. Sodium pentobarbitone anaesthesia (60 mg/kg intraperitoneal) Nerve to area 1 found in the angle between right common carotid and subclavian arteries. Oscillographic records from a few-fibre preparation of nerve to area 1. *A*: control (femoral artery B. P.: 100 mmHg); *B*: haemorrhage of 50 ml of blood (B. P. 30 mmHg); *C*: replacement of the blood (B. P. 80 mmHg).

At the end of the experiments, dissection of the nerve to area 1 down its course in the angle between the right common carotid and the right subclavian arteries showed fine nervous filaments ending in the arterial wall and others in one or two masses of glomus tissue resembling a small carotid body in their gross appearance. One or two similar formations have also been observed in the concavity of the right subclavian artery in the cases in which chemoreceptor activity had been detected in nerve to area 1′. Histology confirmed the epithelioid structure of these tissue formations.

Discussion

Our results support the view that the glomus tissue aggregates, situated in the vicinity of the root of the right subclavian artery, have a chemoreceptor function.

The main part of these aggregates is situated in the angle between the right common carotid and right subclavian arteries and is the body described by Muratori (15-18) and by Nonidez (20); another part has been occasionally found in the concavity of the right subclavian artery and it is the homologous of the body described by Boyd (5) in human embryos.

These aggregates have long been attributed, together with the other similar formations situated in the cardio-aortic area, to the class of the aortic bodies. On the basis of topographic criteria we have suggested the term " right subclavian body " for the glomus tissue aggregates which have been the subject of our study, leaving the term " aortic body " for those situated in the concavity of the aortic arch.

Comroe (6), Gernandt (8), Neil, Redwood and Schweitzer (23) were unable to demonstrate chemoreceptor reflexes from the subclavian body. The electroneurological techniques used in our work provide a much more sensitive method of detecting chemoreceptor activity from small aggregates of chemoreceptor tissue than does the reflex respiratory response to lobeline and other drugs used by previous authors.

The earlier techniques, although capable of detecting chemoreceptor activity from the carotid and the aortic bodies (by demonstrating reflex responses therefrom) were not adequate to show the initiation of reflexes from the small mass of glomus tissue found in the right subclavian body. The detection of chemoreceptor impulses taking origin from this body proved to be more difficult technically than was recording chemoreceptor impulses from the right carotid body under the same conditions. The high density of baroreceptor fibres in the nerve to area 1 renders the detection of chemoreceptor activity difficult. Only in some of the few fibre preparations of the nerve was it possible to detect such activity.

The nerve to baroreceptor area 1′ occasionally contains a few chemoreceptor fibres which represent only a small part of the total chemoreceptor activity from the subclavian body. The finding of chemoreceptor activity in nerve to area 1′ has been associated with the histological finding of additional glomus tissue in the concavity of the subclavian artery.

The evidence that the right subclavian body is chemoreceptor in function, gives further support to the " gill-arch " theory of the evolution of chemoreceptors and baroreceptors (13, 18, 19). According to this hypothesis, the anatomical sites of the baroreceptor and chemoreceptor areas in the mammal are those which survived of the embryonic branchial arch system. The carotid sinus and body regions represent the persisting part of the third arches; the region of the aortic arch and body represents the fourth left embryonic arch. The region of the proximal part of the right subclavian artery and body represents the fourth right embryonic arch. Whereas the other glomus tissue stations have long been known to be chemoreceptor in function, the glomus tissue lying in this region has until now awaited this correlation between its structure and chemoreceptor function.

SUMMARY

The activity of the glomus tissue in the vicinity of the root of the right subclavian artery has been investigated with electroneurographic technique. The results have shown that such epithelioid aggregates have a chemoreceptor function.

Correlation is now possible between structure and chemoreceptor function for the glomus tissue in this region – for which the term " right subclavian body " is suggested – as it has long been for the glomus tissue in the aortic and in the carotid sites.

ACKNOWLEDGMENT

We express our thanks to Mr. P. Gould, Department of Anatomy, Middlexes Hospital Medical School, for the histological preparations.

REFERENCES

1. Bianconi, R. and Green, J. H. Chemoreceptor impulse activity from the right subclavian body of the cat. *J. Physiol.*, *143*: 55-56 P, 1958.
2. Bianconi, R. and Green, J. H. Decorso intratoracico di fibre chemocettive provenienti dai glomi aortici, nel gatto. *L'Ateneo Parmense*, *29*, suppl. *7*: 160-161, 1958.

3. Bianconi, R. and Green, J. H. Baroreceptor innervation of the bifurcation of the brachio-cephalic trunk in the cat. *Arch. ital. Biol.*, *97*: 47-52, 1959.

4. Bogue, J. Y. and Stella, G. Afferent impulses in the carotid sinus nerve (nerve of Hering) during asphyxia and anoxaemia. *J. Physiol.*, *83*: 459-465, 1935.

5. Boyd, J. D. The development of the human carotid body. *Contr. Embryol. Carneg. Instn.*, *26*: 1-31, 1937.

6. Comroe, J. The location and function of the chemoreceptors of the aorta. *Amer. J. Physiol.*, *127*: 176-191, 1939.

7. Diamond, J. and Howe, A. Chemoreceptor activity in the aortic bodies of the cat. *J. Physiol.*, *134*: 319-326, 1956.

8. Gernandt, B. E. A study of the respiratory reflexes elicited from the aortic and carotid bodies. *Acta physiol. scand.*, *11*, suppl. *35*: 1-81, 1946.

9. Green, J. H. Further baroreceptor areas associated with the right common carotid artery in the cat. *J. Physiol.*, *123*: 41-42 P, 1954.

10. Heymans, C., Bouckaert, J. J. et Dautrebande, L. Sinus carotidien et reflex respiratoires. III. Sensibilité des sinus carotidiens aux substances chimiques. Actions stimulante respiratoire du sulfure du sodium, du cyanure de potassium, de la nicotine et de la lobeline. *Arch. int. Pharmacodyn.*, *40*: 54-91, 1931.

11. Heymans, C. and Neil, E. *Reflexogenic Areas of the Cardiovascular System.* London, Churchill Ltd., 1958, VIII-271 pp.

12. Howe, A. The vasculature of the aortic bodies in the cat. *J. Physiol.*, *134*: 311-318, 1956.

13. Koch, E. *Die reflektorische Selbsteuerung des Kreislaufes.* Leipzig, Steinkopff, 1931, x-234 pp.

14. Landgren, S. and Neil, E. Chemoreceptor impulse activity following haemorrhage. *Acta physiol. scand.*, *23*: 158-167, 1951.

15. Muratori, G. Connessioni tra tessuti paragangliari e zone recettrici aortiche. *Atti Soc. med.-chir. Padova*, *12*: 504-509, 1934.

16. Muratori, G. Zona recettrice aortica e tessuto paragangliare. *Boll. Soc. ital. Biol. sper.*, *9*: 1041-1044, 1934.

17. Muratori, G. Connessioni tra tessuto paragangliare e zone recettrici aortiche in vari mammiferi. *Monit. zool. ital.*, *45*: 300-310, 1935.

18. Muratori, G. Osservazioni istologiche e considerazioni embriologiche sui recettori aortici degli amnioti. *Anat. Anz.*, *83*: 367-379, 1937.

19. Muratori, G. Contributi morfologici allo studio dei recettori aortico-arteriosi dei riflessi cardiopressoregolatori. *Arch. ital. Anat.*, *38*: 387-427, 1937.

20. Nonidez, J. F. The aortic (depressor) nerve and its associated epithelioid body, the glomus aorticum. *Amer. J. Anat.*, *57*: 259-293, 1935.

21. Nonidez, J. F. Distribution of the aortic nerve fibers and the epithelioid bodies (supracardial paraganglia) in the dog. *Anat. Rec.*, *69*: 299-313, 1937.

22. Neil, E. Chemoreceptor areas and chemoreceptor circulatory reflexes. *Acta physiol. scand.*, *22*: 54-65, 1951.

23. Neil, E., Redwood, C. R. M. and Schweitzer, A. Effects of electrical stimulation of the aortic nerve on blood pressure and respiration in cats and rabbits under chloralose and nembutal anaesthesia. *J. Physiol.*, *109*: 392-401, 1949.

Arch. ital. Biol., *98*: 1-9, 1960.

RECHERCHES SUR L'ÉPILEPSIE EXPÉRIMENTALE FOCALE PROVOQUÉE PAR L'APPLICATION DE PÉNICILLINE SUR L'ÉCORCE CÉRÉBRALE

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Introduction

Les accès généralisés observés chez l'homme ont souvent leur origine dans un foyer cortical limité. Malgré de nombreuses recherches effectuées dans ce domaine, le mécanisme grâce auquel une décharge focale conduit à un accès généralisé n'est pas suffisamment connu.

Après avoir étudié le déroulement de l'accès jacksonien, les cliniciens ont attribué à l'écorce cérébrale le rôle essentiel dans la propagation des décharges focales. Celles-ci se propageraient de proche en proche sur le cortex jusqu'à l'invasion totale de ce dernier. Ultérieurement, les recherches expérimentales démontrèrent que la propagation des décharges épileptiques, loin d'être une simple extension en tache d'huile sur la surface de l'écorce cérébrale, se fait en suivant certaines voies anatomiques bien définies. Moruzzi (8) a démontré que l'activité convulsive provoquée par la strychnine appliquée sur un point du cortex, envahit l'aire homologue de l'hémisphère controlatéral avant de se propager sur toute autre formation corticale. Ensuite il devint de plus en plus évident que des formations souscorticales jouent un rôle dans la propagation des décharges focales et on discuta l'intervention de circuits révérbérants corticosouscorticaux.

L'attention a été d'abord attirée par les connexions thalamocorticales et cortico-thalamiques. L'activité convulsive des formations extrapyramidales a été étudiée par Moruzzi (9, 10) sur des chats chloralosés et ayant subi une injection de strychnine. Parmi les recherches plus récentes concernant le rôle des formations sous-corticales dans la propagation des décharges corticales il faut citer celles d'Ajmone Marsan et Stoll (1) et de Walker, Poggio et Andy (13).

Dans la présente étude nous nous sommes proposé d'explorer chez le chat le mécanisme de la propagation des décharges issues de l'aire sensitivo-motrice (gyrys sigmoïdien). L'importance de cette zône comme point de départ de certaines crises convulsives est bien connue et elle a été récemment soulignée par Penfield et Jasper (11). Ces auteurs affirment que dans les tumeurs cérébrales l'incidence des accès diminue d'autant plus que la distance entre la tumeur et l'écorce motrice augmente.

MÉTHODES

Pour créer un foyer épileptogène, nous avons appliqué de la pénicilline sur l'écorce cérébrale, méthode employée par de nombreux auteurs, dont Walker *et al.* (13). Nos recherches ont été effectuées sur 40 chats curarisés et non anesthésiés. Les cristaux de pénicilline étant placés sur le cortex, nous avons dérivé l'activité électrique de différentes formations corticales ou souscorticales à l'aide d'électrodes bipolaires implantées dans ces formations. La distance entre les pôles d'une électrode est de 1,5 mm. Les animaux sont sacrifiés après l'expérience, les cerveaux fixés et la localisation des électrodes déterminée macroscopiquement.

RÉSULTATS

Immédiatement après l'application locale de penicilline, il se produit au niveau du foyer une augmentation de la fréquence et une diminution de l'amplitude des ondes cérébrales. Le même phénomène a été observé par les auteurs utilisant la strychnine appliquée sur l'écorce cérébrale (4). Des fuseaux d'ondes qui apparaissaient périodiquement sur toutes les dérivations disparaissaient au niveau du foyer. Kornmüller (7) et Janzen, Magun et Bechter (5) avaient déjà signalé ce phénomène. Depuis l'application de la pénicilline jusqu'à l'apparition des premières décharges épileptique il y a un intervalle de 2-4 minutes. Ces premières décharges ont un voltage réduit et consistent en simples pointes mono -ou biphasiques. Plus tard l'amplitude augmente, ainsi que la fréquence et la complexité de ces décharges (Fig. 1). Des ondes viennent s'ajouter aux pointes et forment parfois des complexes pointe-onde. Puis les décharges se compliquent, elles sont formées d'une grande pointe suivie de plusieurs petites pointes; parfois on observe des complexes polypointes-onde. Les accidents, isolés au début, séparés par un intervalle de quelques secondes deviennent de plus en plus rapprochés, se groupent par deux et puis, lorsque l'accès est généralisé, les décharges sont continues, rythmiques, plusieurs par seconde.

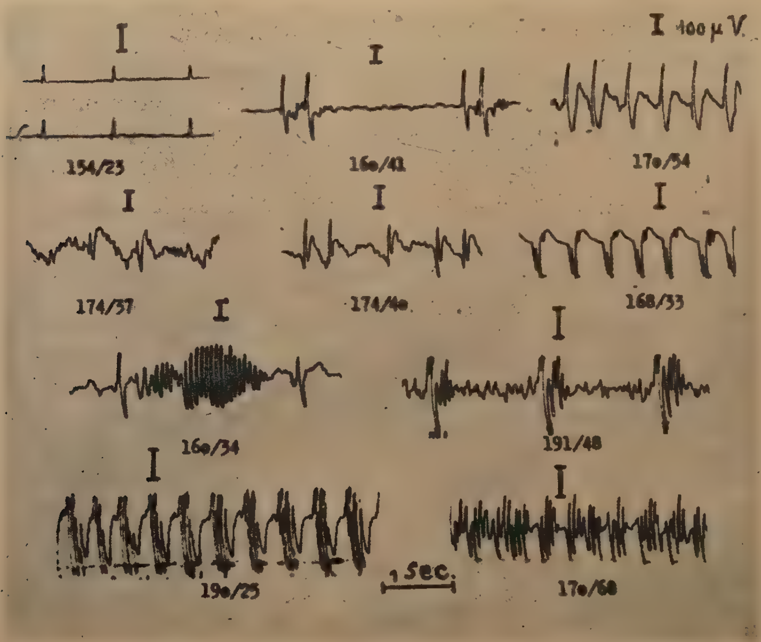

Fig. 1. – *Différents types de décharges focales après l'application de pénicilline sur le gyrus sigmoïdien du chat.*

Nous avons tenté de vérifier s'il y a une influence réciproque entre l'activité électrique de base du cerveau et l'activité du foyer convulsif, comme l'ont affirmé Hippius, Rosenkätter et Sellbach (4). Ces auteurs, expérimentant sur le lapin après application locale de strychnine sur l'écorce cérébrale, ont observé que l'activité du foyer convulsif était contrôlée par le niveau de l'activité éléctrique de fond. Tant que celle-ci était constituée par des ondes lentes, l'activité focale était formée de pointes isolées; par contre, la desynchronisation de l'activité basale était suivie par l'apparition de salves de pointes. Nous n'avons pu constater ces phénomènes dans nos con-

tions d'experience et nous avons observé une indépendance entre
s décharges focales et l'activité de fond.

Propagation des décharges provoquées par l'application de péni-
lline sur le gyrus sigmoïde. — Les premières décharges produites
ar la pénicilline ont un voltage réduit et ne sont pas propagées à
istance. A mesure que l'amplitude des décharges augmente, l'acti-
ité paroxystique s'étend à des structures de plus en plus nombreuses
usqu'à ce qu'elle se généralise. Nous avons tenté de préciser les
oies de cette propagation et c'est pourquoi nous nous sommes pro-
osé de déterminer les formations anatomiques qui deviennent les
remières le siège d'une activité convulsive. A l'aide d'un électro-
ncéphalographe à huit canaux dont l'un dérivait l'activité du foyer
rimaire et les autres celle de différentes formations situées à dis-
ance, nous avons précisé pour chaque expérience les 3 formations
ui les premières devenaient le siège d'une activité paroxystique.
n plus, nous avons noté les formations qui participaient en retard
la propagation des décharges. En totalisant les résultats obtenus
ur 40 chats nous avons pu tirer certaines conclusions sur l'ordre
ans lequel différentes formations sont envahies par l'activité con-
ulsive.

La propagation vers les noyaux ventraux du thalamus est pré-
oce et importante (Figs. 2 et 3). Sur 21 enregistrements des noyaux
entraux, la propagation a été précoce dans 19 cas. Ce phénomène
été enregistré tant dans les noyaux ventraux latéraux (10 propa-
ations précoces, 1 incertaine) et ventraux postérieurs (3 cas à pro-
agation précoce, 1 cas à progagation tardive), que dans le noyau
entral antérieur faisant partie du système diffus de projection
4 enregistrements démontrant tous une propagation précoce).

La propagation précoce vers le noyau dorsomédial du thalamus,
quoique moins constante (7 propagations précoces sur 11 dériva-
ions), reste pourtant fréquente. Il faut signaler que le noyau para-
asciculaire devient précocement le siège d'une intense activité co-
mitiale propagée (5 cas des 6 étudiés).

La propagation vers les noyaux latéraux du thalamus n'est pas
fréquente. Sur 19 enregistrements, 4 seulement ont présenté une
propagation précoce. En ce qui concerne l'activité individuelle des
différents noyaux du groupe latéral, nous constatons: *a*) noyau la-
téral postérieur: 8 enregistrements, aucune propagation précoce;
b) noyau latéral dorsal: 8 enregistrements, 3 propagations précoces;

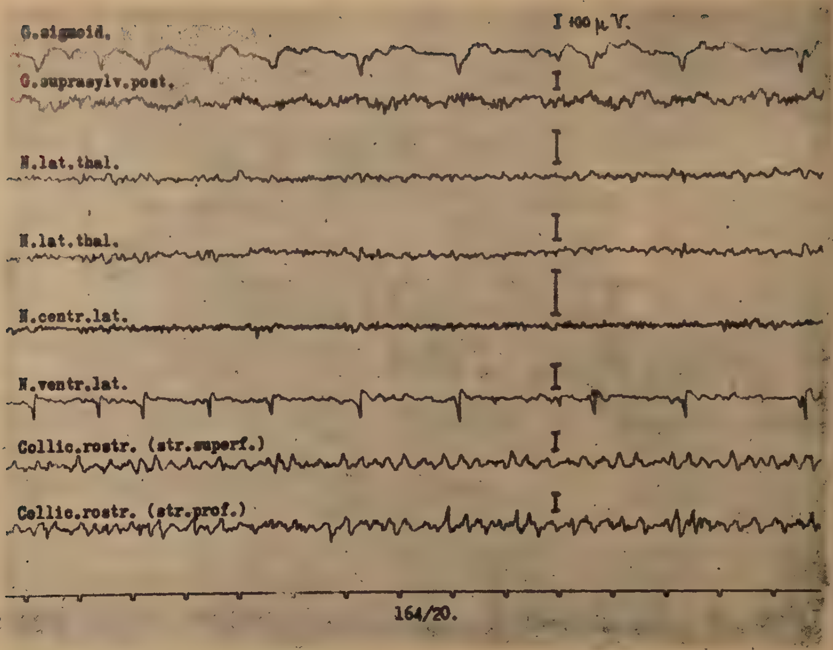

Fig. 2. – *Foyer pénicillinique au niveau du gyrus sigmoïdien avec décharges synchrones limitées au noyau ventro-latéral du thalamus.*

Dans cette figure et dans les suivantes le temps est une seconde.

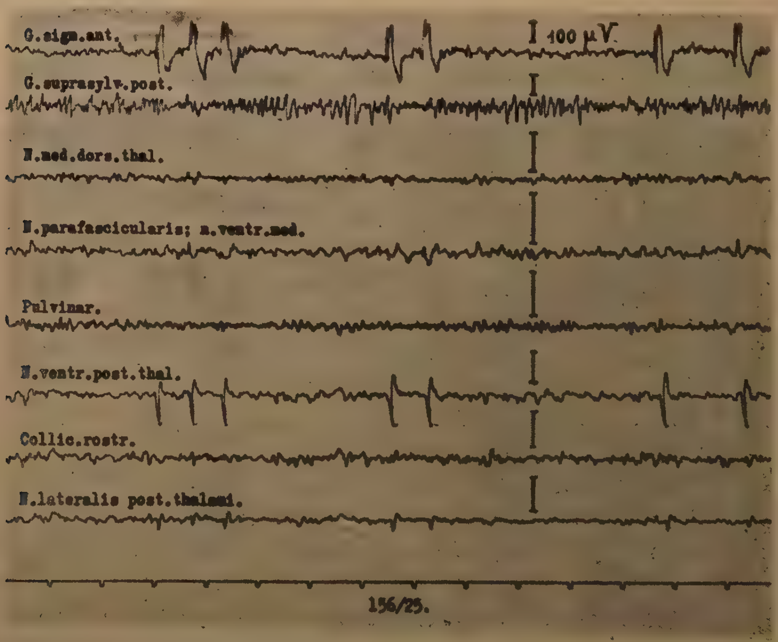

Fig. 3. – *Foyer pénicillinique au niveau du gyrus sigmoïdien antérieur avec décharges synchrones limitées au noyau ventral postérieur du thalamus.*

noyau réticulaire: 3 dérivations, 1 propagation précoce. De plus faut citer dans ce même groupe le pulvinar (3 cas, aucune propa-tion précoce).

Le groupe des noyaux de la ligne médiane ne participe pas à propagation: un seul cas précoce sur 10 dérivations. Le noyau udé non plus ne présente pas d'activité comitiale précoce: 6 cas gatifs, 1 précoce.

Parmi les formations du mésencéphale, nous avons étudié la bstance réticulée (7 propagations précoces sur 12 dérivations) et tubercules quadrijumeaux antérieurs (5 propagations précoces r 22 dérivations).

Le putamen, considéré par de nombreux auteurs comme l'une s étapes les plus importantes de la propagation des décharges sues du cortex noteur, n'a pas été étudié dans ce travail.

Quelle est la participation de l'écorce cérébrale au processus de opagation des décharges focales? Dans nos expériences nous avons servé que l'extension des décharges vers le gyrus sigmoïde du té opposé est relativement précoce (6 cas sur 10 dérivations). Il ut pourtant remarquer que la propagation d'un gyrus sigmoïde à utre ne s'effectue que lorsque toute une série de formations sous-rticales, noyaux du thalamus surtout, sont déjà envahies par les charges paroxystiques. Quant à la propagation vers l'écorce po-érieure, elle est très tardive, ne se produisant qu'après l'invasion s noyaux thalamiques (Fig. 4). Sur 22 enregistrements des *gyri prasylvius*, *ectosylvius* et *lateralis* il n'y a que 4 propagations écoces.

La propagation des décharges issues du gyrus sigmoïde vers ippocampe est difficile à caractériser, le nombre des résultats sitifs, négatifs et douteux étant à peu pres égal.

Nous avons en plus étudié l'effet de l'asphyxie sur les décharges roxystiques déterminées par la pénicilline. A cet effet nous avons rêté la pompe à respiration artificielle pendant 2-4 minutes. L'acti-té électrique convulsive s'arrêtait après une asphyxie de la durée oyenne de 85 secondes calculée sur 15 expériences (valeurs extrêmes et 130 secondes).

La disparition de l'activité convulsive au caus de l'asphyxie ait en général progressive: les décharges devenaient de plus en us rares, le voltage des accidents diminuait et les décharges com-lexes composées de plusieurs oscillations devenaient de plus en plus mples. D'habitude l'activité électrique épileptique disparaît en même temps dans toutes les formations. On observe pourtant parfois une persistance des décharges dans certaines formation sous-corticales après l'arrêt de l'activité corticale. C'est ainsi que nous avons pu observer chez un animal la persistance des décharges paroxystiques, pendant quelques secondes, dans la substance grise périaqueductale (Fig. 5) et chez deux autres animaux, dans les noyaux du thalamus.

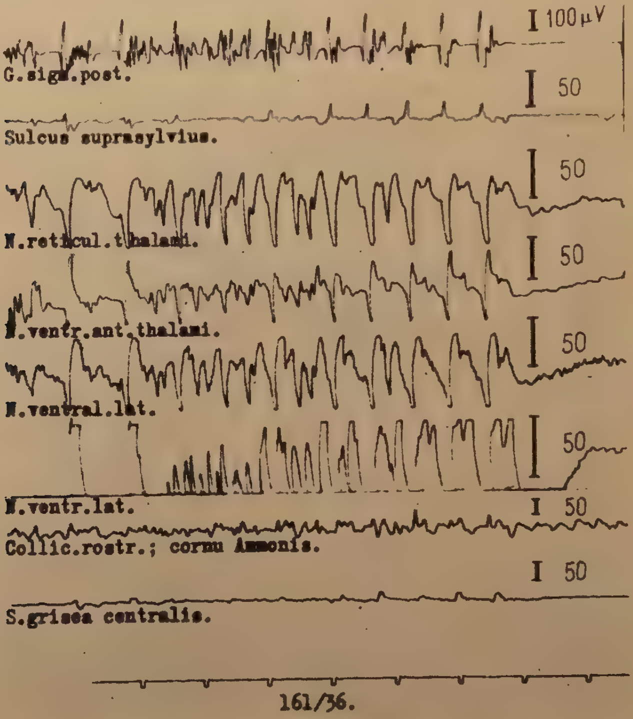

Fig. 4. – *Foyer pénicillinique au niveau du gyrus sigmoïdien postérieur avec décharges propagées aux noyaux thalamiques.*

Les décharges sont tardives et faibles sur le cortex suprasylvien.

La reprise de la respiration artificielle après un arrêt de 2 minutes fait réapparaître les décharges paroxystiques après un intervalle moyen de 95 secondes (les valeurs extrêmes sont 17 et 240 secondes). Dans la plupart des cas l'apparition de l'activité convulsiv est précédée par l'apparition des fuseaux d'ondes. En général l'activité paroxystique se rétablit d'abord au niveau du foyer primitif ans certains cas pourtant, les premières décharges apparaissent ans d'autres formations. C'est ainsi que chez 2 animaux chez lesquels ous avions appliqué la pénicilline sur le gyrus latéral et suprasylien, les premières décharges, après la reprise de la respiration, pparurent sur le gyrus sigmoïde. Dans d'autres cas, où la pénicilline

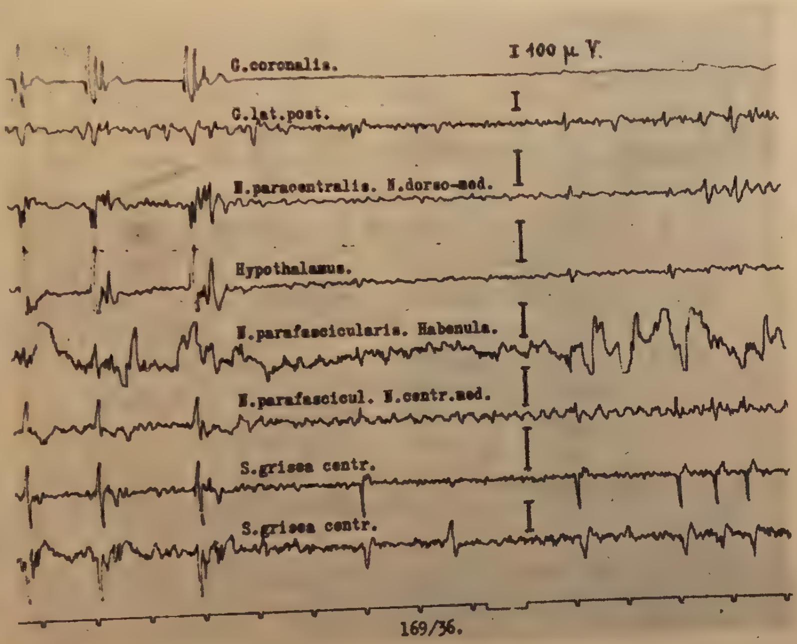

Fig. 5. – *Effet de l'asphyxie sur l'activité convulsive entraînée par un foyer pénicillinique du gyrus sigmoïdien.*

Asphyxie pendant une minute. Suppression des décharges corticales et thalamiques. Les décharges persistent dans la substance grise périaquéductale.

avait été appliquée sur le gyrus sigmoïde, ce sont des formations sous-corticales (noyau parafasciculaire, noyaux de la ligne médiane, noyau ventral latéral) qui ont présenté les premières décharges paroxystiques après la fin de l'asphyxie.

La chlorpromazine, jusqu'à 12 mg/kg n'a eu aucune action évidente sur l'accès focal pénicillinique. On n'a pas observé de modifications de l'activité du foyer primitif; de même on n'a pas enregistré une influence quelconque de la chlorpromazine sur l'activité propagée.

DISCUSSION

Il faut remarquer que nos résultats ne sont pas comparables à ceux de la neuronographie physiologique. Dans la neuronographie on utilise des foyers discrets d'excitation et on admet généralement que les décharges sont incapables de dépasser le premier synapse rencontré. Dans notre cas il s'agit des décharges d'un puissant foyer épileptogène, traversant les synapses les uns après les autres jusqu'à la généralisation de l'accès convulsif. Le fait de démontrer l'existence d'une voie réunissant deux centres nerveux n'implique pas forcément que cette voie sert à la propagation d'un accès d'épilepsie. C'est ainsi que French, Gernandt et Livingstone (2) ont démontré que malgré les riches connexions du cortex orbitaire et para occipital avec le système centrencéphalique, l'excitation de ces formations n'a que de faibles effets épileptogènes. Il ne suffit donc pas que des connexions anatomiques existent, il faut aussi qu'elles possèdent certaines caractéristiques physiologiques pour propager un accès convulsif. En effet, ainsi que l'affirment les auteurs cités, certaines des connexions conduisent des effets inhibiteurs, d'autres donnent naissance à des post-décharges puissantes.

Dans leurs expériences, Walker, Poggio et Andy (13), à l'aide d'excitations focales électriques et d'applications focales de pénicilline ont précisé chez le singe le mode de propagations des décharges parties de différentes régions corticales. En ce qui concerne les décharges ayant comme point de départ le cortex sensorimoteur (gyrus pré- et postcentral), ils décrivent les propagations préférentielles suivantes: 1) cortex central hétérolatéral; 2) putamen homolatéral, intéressé dans 90% des cas; 3) noyaux latéraux du thalamus ayant une activité convulsive dans 30% des cas; 4) noyau sousthalamique, locus niger, substance réticulaire pontine. Ils n'en ont pas observé dans le noyau caudé, l'hypothalamus, l'hippocampe et l'amygdale. Hayashi (3) et Jinnai *et al.* (6) avaient déjà souligné l'importance du putamen dans la propagation des décharges issues du cortex moteur et préconisé la lenticulotomie dans le traitement de l'épilepsie.

Nos recherches sur la propagation des décharges issues de la region sigmoïdienne nous ont permis de constater que: 1) les premières formations envahies par l'activité paroxystique sont les noyaux ventraux latéral et postérieur du thalamus et la formation réticulée du mésencéphale; 2) les noyaux dorso-médial et parafasciulaire sont interessés plus tard; 3) les noyaux de la ligne médiane, es noyaux latéraux et le pulvinar, sont envahis encore plus tard, eu de temps avant la généralisation de l'accès; 4) enfin, en dernièr eu, les décharges se propagent vers le cortex postérieur (gyrus ateralis et suprasylvius).

Le rôle respectif de l'écorce cérébrale et des structures souscorticales dans la propagation de l'activité épileptogène n'est pas tout fait élucidé. Dans des recherches sur l'accès convulsif provoqué par l'injection intraveineuse d'une dose liminaire de métrazol, Starzl, Wiemer, Dell et Forgrave (12) ont démontré que l'activité convulsive des différents noyaux thalamiques est strictement dépendente de l'activité des régions corticales en connexion avec ces noyaux. C'est ainsi que l'activité paroxystique du noyau latéral postérieur ne fait que refléter l'activité du cortex latéral ou suprasylvien moyen. Ces auteurs considèrent que dans le cas des doses liminaires de métrazol, l'activité des noyaux thalamiques dépent on premier rang des impulsions corticifuges. Ainsi que nous l'avons montré plus haut, nous avons observé, dans nos expériences avec foyer pénicillinique, des décharges paroxystiques intenses dans des noyaux thalamiques dont les zones de projection corticale ne présentaient aucune activité convulsive. Nous considérons que les décharges parties du foyer cortical sigmoïdien se propagent d'abord dans les noyaux de projection de la région sigmoïdienne, noyaux ventraux latéral et postérieur du thalamus et la formation réticulée du tronc cérébral. C'est ici que s'établissent des circuits internucléaires qui conduisent à une généralisation de l'activité convulsive au niveau des noyaux du thalamus et plus tard à l'écorce toute entière.

RÉSUMÉ

Le mécanisme de généralisation de l'activité convulsive est étudié à l'aide d'un foyer épileptogène primaire produit par l'application de pénicilline sur le cortex sigmoïde du chat. Les noyaux ventraux latéral et postérieur du thalamus et la formation réticulée du mésencéphale sont les premiers envahis par l'activité paroxystique. Dans une phase plus avancée, l'activité convulsive peut être dérivée du noyau dorso-médial. La participation des noyaux latéraux, de ceux de la ligne médiane et du pulvinar est tardive, elle précède de peu la généralisation. L'écorce cérébrale postérieure est la dernière à être envahie par l'activité convulsive.

Par conséquent, la généralisation d'un accès convulsif focal sigmoïdien n'est pas produite par sa propagation corticale, mais par un envahissement progressif d'une large portion des structures souscorticales.

BIBLIOGRAPHIE

1. Ajmone Marsan, C. and Stoll, J. Subcortical connexions of the temporal lobe in relation to temporal lobe seizures. *Arch. Neurol. Psychiat., Chicago, 66*: 669-686, 1951.
2. French, J. D., Gernandt, B. E. and Livingston, R. B. Regional differences in seizure in monkey cortex. *Arch. Neurol. Psychiat., Chicago, 75*: 260-272, 1956.
3. Hayashi, T. A. A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. *Jap. J. Physiol., 3*: 46-64, 1952.
4. Hippius, H., Rosenkätter, L. and Sellbach, H. Untersuchungen zur Verlaufsdiagnostik korticaler Krampfpotentiale. *Arch. Psychiat. u. Z. ges. Neurol., 196*: 379-401, 1957.
5. Janzen, R., Magun, R., Bechter, F. Tierexperimentelle Untersuchungen über die Ausbreitung der Epileptischen Erregung. *Dtsch. Z. Nervenheilk., 166*: 223-237, 1951.
6. Jinnai, D., Yoshida, T., Souji, T. and Kosaka F. Experimental studies on the march of spasms during epileptic convulsions. *Acta med. Okayama, 8*: 26-29, 1950.
7. Kornmüller, A. E. *Die bioelektrischen Erscheinungen der Hirnrindenfelder.* Leipzig, Thieme, 1937, 118 pp.
8. Moruzzi, G. Contribution à l'électrophysiologie du cortex moteur: facilitation, afterdischarge et épilepsie corticales. *Arch. int. Physiol., 49*: 33-100, 1939.
9. Moruzzi, G. Convulsioni extrapiramidali ottenute mediante iniezioni endovenose di piccole dosi di stricnina nell'animale in narcosi cloralosica. *Boll. Soc. ital. Biol. sper., 16*: 167-169, 1941.
10. Moruzzi, G. Convulsioni extrapiramidali da stricnina. *Arch. Fisiol., 44*: 109-162, 1945.
11. Penfield, W. and Jasper, H. *Epilepsy and Functional Anatomy of the Human Brain.* Boston, Little, Brown and Co., 1954, xv-896 pp.
12. Starzl, T. L., Niemer, W. T., Dell, M. and Forgrave, P. R. Cortical and subcortical electrical activity in experimental seizures induced by metrazol. *J. Neuropath., 13*: 262-276, 1953.
13. Walker, A. E., Poggio, G. F. and Andy, O. J. Structural spread of cortically induced epileptical discharges. *Neurology, 6*: 616-626, 1956

Arch. ital. Biol., 98: 10-20, 1960.

MICROELECTRODE INVESTIGATIONS ON THE MECHANISMS OF THE ELECTRICALLY INDUCED EPILEPTIFORM SEIZURE (" AFTERDISCHARGE ")[1]

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INTRODUCTION

Since the early descriptions by Adrian (1) and Rosenblueth and Cannon (26), many authors have studied the rhythmical self-sustained activity elicitable with repetitive electrical stimulation of the cerebral cortex. This activity, often called " afterdischarge ", is very similar to the spontaneous " electrical " seizures recordable from the exposed cortex of epileptic patients and has been extensively used as a tool to experimentally analyze epileptic phenomena. Notwhitstanding the number of investigations, however, we still ignore its intimate mechanisms. On the basis of different findings, several hypotheses have been advanced.

Forbes (17) was among the first to suggest the existence of " reverberating " circuits by which each cell may contribute to the continuation of its own activity.

Adrian and Rosenblueth and Cannon explain the afterdischarge by the " intrinsic ability of some cortical cells to fire repetitively during a period of excitation ".

The problem of the development of self-sustained activity was later approached by different methods. Several authors (6, 20, 24) have analyzed the modifications of the cortical responses during repetitive stimulation capable of producing afterdischarge. They describe a progressive decrease in amplitude and eventual inversion of polarity and disappearance of the responses, and subsequently the appearance of repetitive activity; afterdischarge may follow even if the stimulation is arrested at the first stages.

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Jung (20) suggests an ingenious, though purely theoretical hypothesis: the slow, initial negative response to each stimulus should be considered the expression of a " braking " mechanism in the absence of which convulsive activity takes place. Bremer (8) invokes the development of " complex chains of processes of synaptic facilitation and of post-reactional depression " in which " the residual membrane depolarizations constitute the endogenous stimulus for the self-sustained activity ".

By recording transcortical *DC* potentials, and by interpreting them in relation to the particular histological features and orientation of the giant pyramidal cells, King *et al.* (24) and Burns (9, 10) independently demonstrate the presence of a small surface positivity during the afterdischarge. Burns interprets it as the expression of a difference of polarization between dendrites and cell body and considers this difference the necessary and sufficient condition for the occurrence of self-sustained activity. Similar conclusions were also reached by Green *et al.* (15) working with different technique in the hippocampus.

Unitary phenomena during afterdischarges were studied as early as 1939 (2). More recently, since the introduction of the microelectrode technique, a few studies became available chiefly from Jung's school (4, 5, 11, 20, 21, 22) without, however, contributing to the understanding of the mechanisms at the basis of the development of the afterdischarge.

In this paper we report the results of systematic microelectrode exploration of the cellular phenomena characterizing development and end of the electrical afterdischarge and offer an interprepation of the mechanism at the basis of this type of activity.

METHODS

Experiments, all acute, were performed on curarized cats. Only at the beginning of the experiment small doses of pentothal were used for vein incanulation, tracheotomy and craniotomy (except for 2 animals on which ether and *cerveau isolé* preparation were used). Recording and stimulation were then carried out under artificial respiration. Craniotomy was bilateral and rather extensive, but the dura was opened only on a small area overlying the suprasylvian gyrus. In order to drain the cranial fluid continuously and thus establish a better stability of the brain, a posterior fossa craniectomy was also carried out with opening of dura and arachnoid above the 4th ventricle.

Cortical stimulation through bipolar silver electrodes 1 mm apart, was performed with a Grass model S 4 stimulator and isolation unit. Its parameters were varied (see Results) but the optimal stimulation for elicitation of afterdischarge consisted of 3 sec trains of square waves of 1 msec duration, 10 V at 50 sec with at least 1 min intervals between subsequent trains.

Pick-up of the cortical electrical activity was by means of silver-silver chloride electrodes placed about 1 mm away from the stimulating ones over

Fig. 1. – *Comparative sizes of a microelectrode tip and of a giant pyramidal cell of the suprasylvian gyrus.* Scale = 20 μ.

the suprasylvian gyrus and also on a symmetrical point in the homologous area of the opposite hemisphere; the frame of the stereotaxic instrument was used as reference electrode. Tungsten microelectrodes (19) were used, their movement being controlled by a hydraulic micromanipulator. The size of the tip was about 2 μ (see Fig. 1) and their resistance approximately 10-20 MΩ. These were inserted perpendicularly to the surface of the cortex as much as possible in the middle of the gyrus, close to the electrodes for stimulation and EEG pick-up. Cortical surface and unitary records were displayed by means of an Offner 8 channel ink-writing machine and of a double beam CRO (DuMont type 333) after amplification through two Tektronix type 122 preamplifiers, using a cathode follower (3) for the microelectrode. The time constant used was the maximal available in our AC amplifying system except in some instances in which it was purposely reduced for the recording of the unit activity. In the oscillographic records the upper beam is used for the cortical surface activity and the lower beam for the microelectrode recording. Positivity is indicated by downward deflection for the EEG and upward deflection for the microelectrode tracing. The calibration signals in the various illustrations refer only to the lower (microelectrode) beam.

Approximately 2500 afterdischarges were elicited, but only part of these were accompanied by visible spike activity. Thus, films were taken of about 600 afterdischarges with the corresponding activity of 80 different units. Of these, 50 could be kept for over 5 minutes and up to 1 hour. Only these long-lasting cells, characterized by a constant type of behavior throughout most of their recording, were considered in good condition and only these were analyzed in the present paper. Many of them were very often rather " quiet " in resting conditions and could well have been missed in the absence of electrical stimulation. In order to avoid confusion, the word " spike " will never be used here in reference to EEG changes.

Attempts to locate the microelectrode tip by histological methods were done only sporadically and, therefore, no statement concerning the topographical origin of the unit activity will be made in the course of this presentation.

RESULTS

1. *Unit behaviour during electrical stimulation.* — The property, for a given stimulation, to elicit or not an afterdischarge depends on several factors: strength (pulse voltage and duration), frequency of repetition, total duration of the train of repetitive pulses, and, for subsequent stimulations, duration of the interval between the end of the afterdischarge and the beginning of the subsequent stimulation. By varying one of these parameters, it is possible to analyze the unit behaviour when self-sustained activity does, or respectively, does not take place. Examples of this study are seen in Figs. 2 and 3. With inadequate pulse duration (Fig. 2 *B*), voltage (Fig. 2 *D*) and frequency (Fig. 2 E), as well as with too short a train of pulses (Fig. 3 *A*) no afterdischarge follows the stimulation. The effects of respective increase in these parameters when stimulating the same units are shown in Fig. 2 *A*, *C*, *F* and 3 *B*, where an afterdischarge is instead produced (Fig. 3 *C*).

Fig. 2. – *Effects of variation of stimulation parameters* (pulse duration, voltage, frequency).

A, *C*, *F*: efficient stimuli; *B*, *D*, *E*: stimuli inadequate to produce afterdischarge. Successive stimulations of same units in *A*, *B*; *C*, *D*; *E*, *F*. Same train of stimuli; one second interval between each frame. The three graphs schematically illustrate the course of spike amplitude evolution in the different situations. (See text for further details).

Fig. 3. – *Effects of variation of stimulus duration.*

A: no afterdischarge follows a 4 sec stimulation. Note a slight decrease in the amplitude of the spikes elicited with the last 7 pulses. *B*: longer lasting stimulation (8 sec) is followed by an afterdischarge (*C*). Note marked decrease in amplitude and disappearance of spikes during the stimulation and progressive amplitude increase in the course of the afterdischarge. *B*-*C*: each frame is separated by about 1 sec interval and corresponds to the black dots visible in the ink-writing record.

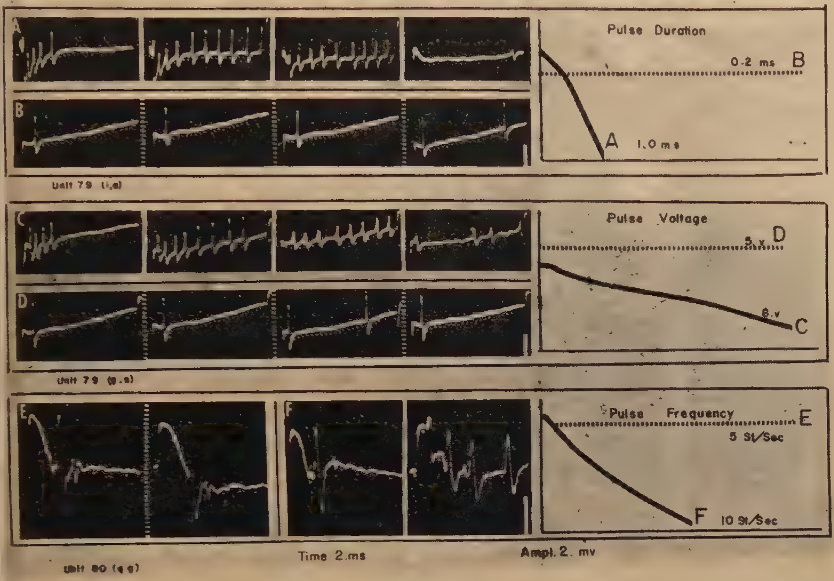
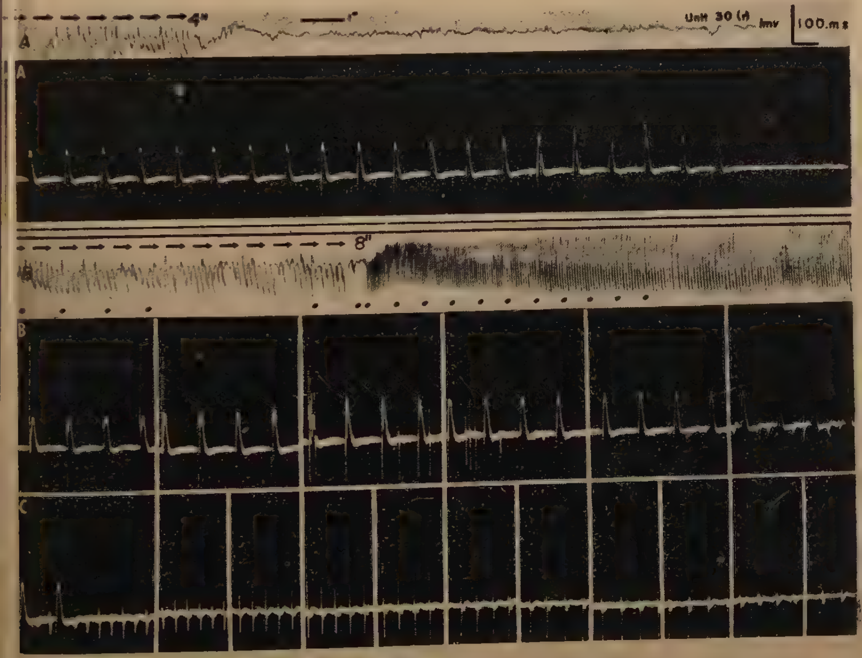

A
B
Unit 79 (i,a)
Pulse Duration
0.2 ms B
A 1.0 ms
C
D
Unit 79 (g.a)
Pulse Voltage
5 v D
8 v
C
E
F
Unit 80 (g g)
Pulse Frequency
E
5 St/Sec
F 10 St/Sec
Time 2.ms
Ampl. 2. mv

Fig. 2.


4"
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1mv
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A
A
8"
B
C

Fig. 3.

With inadequate frequency (less than 10/sec), a weak pulse strength does not elicit any unit response; progressive increase in strength first produces unconstantly a spike, then a single spike follows each stimulus and eventually repetitive response may appear. The amplitude of the spikes does not vary and no afterdischarge

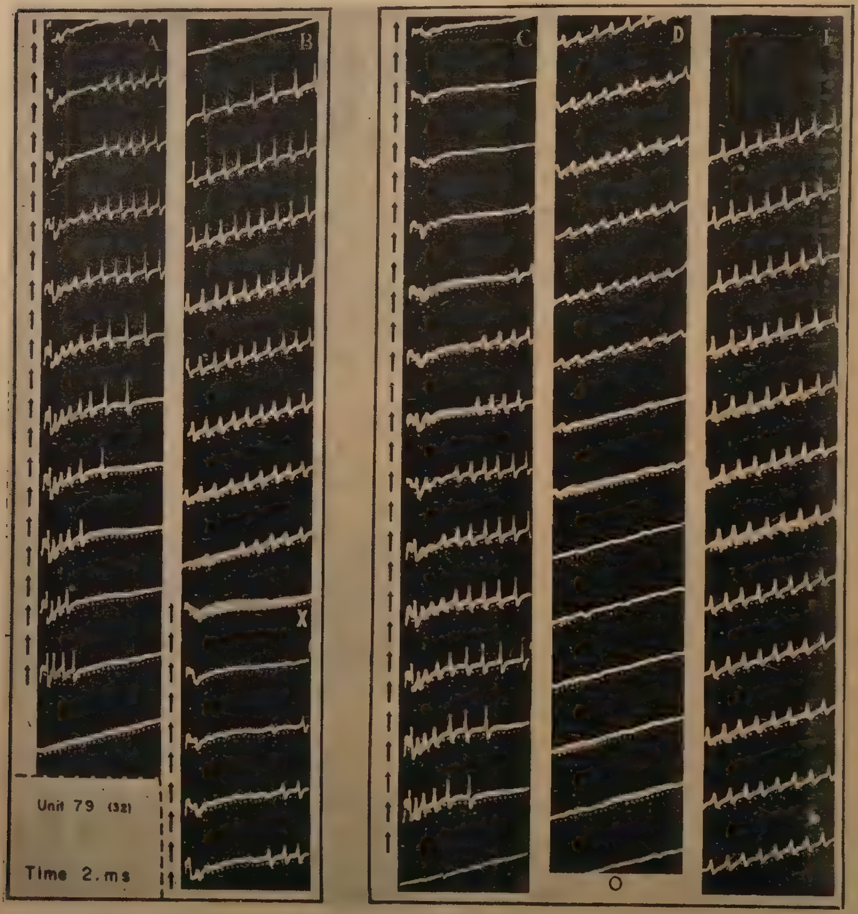

Fig. 4. – *Unit activity during two successive stimulations and the following afterdischarges.*

A, *B*, *C*: show the typical repetitive bursts with their peculiar size evolution (see text). The silent phase is much shorter in *B* (where pulses stop when spikes disappear, in *X*) than in *C* (where the stimulation is continued 1 sec later). There is an interval of 3 sec between *C* and *D* (at *O*). Parameters of stimulation: 8 V, 20/sec, 2.5 msec pulse duration. Records read from bottom to top. Note progressive amplitude increase in the course of the afterdischarge (*B*, *D*, *E*).

appears, (see Fig. 2 *B*, *D*, *E* where, however, the frequency is slightly higher). When the stimulus is strong enough to constantly elicit a spike, a progressive increase in the frequency facilitates the appearance of repetitive firing. The response consists then of a burst of spikes in which the first is the highest, while the second is the smallest and the following progressively increase. But the most typical feature in such cases is the progressive decrease of the absolute spike amplitude in subsequent stimuli (see graphs *A*, *C*, *F* in Fig. 2). This evolution of the burst pattern continues until the total disappearance of the spike; the earliest spikes to disappear are those roughly corresponding to the middle portion of the burst; then the first one and eventually the last spike disappears (Fig. 4). When this stage is reached an afterdischarge will follow.

By progressively increasing the total duration of the train of pulses, it should be possible to evaluate the degree of diminution which the spike amplitude must reach in order for an afterdischarge to develop (in Fig. 3 *A*, for instance, some bursts progressively decreasing in amplitude appear but the total stimulus duration is not long enough to make possible the development of self-sustained activity). Absolute values in this respect were not determined and it can only be stated that a stimulation is always followed by afterdischarge if it was strong and long enough to produce the disappearance of the spike..

2. *Unit behaviour during afterdischarge.* — This silent phase characterized by absence of spikes may be considered analogous to the EEG " latent " period described by Rosenblueth and Cannon (26). Its duration varies from fractions of a second up to several seconds, and depends to a certain extent upon whether the stimulation is interrupted exactly at its very onset or whether it is carried on for a while after the spikes have disappeared (see Fig. 4 *B* at *X* and 4 *D*).

The reappearance of the spikes generally takes place in coincidence with the onset of the " tonic " phase of the EEG discharge (Fig. 5). If, in the course of an afterdischarge we consider only the time of appearance of spikes, regardless of whether they express the activity of a single or more units in the same or in different microelectrode placements, we note that chances of their firing increase progressively with the development of the afterdischarge. Thus, a cell may or may not be activated at the very beginning of the EEG " tonic " phase, but its firing is almost the rule at the time of the " clonic " phase, and when more than one unit is recorded in the same place, it is in the later phases of the afterdischarge that the activity of the largest number of cells becomes manifest (Fig. 6). One may frequently observe a relationship between the apparition of (a new) unit firing and some change in the EEG pattern (see Fig. 5).

The earliest spikes are not always clearly related to the cortical slow waves and may fire irregularly, but very soon they become rhythmical, each spike being synchronized with each of the slow EEG oscillations. This synchrony, however, is not strictly absolute and, particularly when more than one unit is present their spikes tend to fire in bursts whose organization is not exactly similar for the successive slow waves (see Fig. 6). Although the same unit in different afterdischarges tends to have a rather similar behaviour,

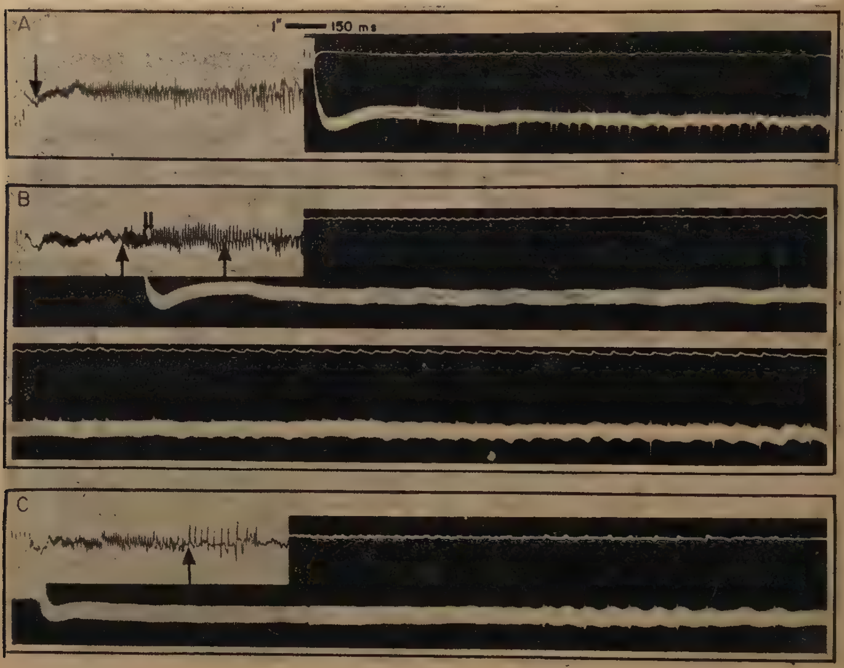

Fig. 5. – *Different delays of onset of unit activity.*

A: the firing is simultaneous with the onset of the clonic phase (arrow). *B*: the small spikes appear (first arrow) late in the tonic phase (note change in the EEG tracing) and are briefly interrupted when the EEG looses this pattern (small arrows). High voltage spikes from a different unit appear much later (last arrow). *C*: firing at the onset of the clonic phase.

numerous examples are seen in which the time of appearance in relation to the EEG pattern varies in subsequent stimulations.

All these findings refer to celles which, according to the criteria mentioned in the Methods, are considered to be in good condition. In the presence of definite injury discharges, or in those cases in which the cell is already actively firing at a high rate in resting conditions, electrical stimulation and afterdischarges do not seem to affect the unit in question or the only visible effect consists of an interruption of its firing (Fig. 7).

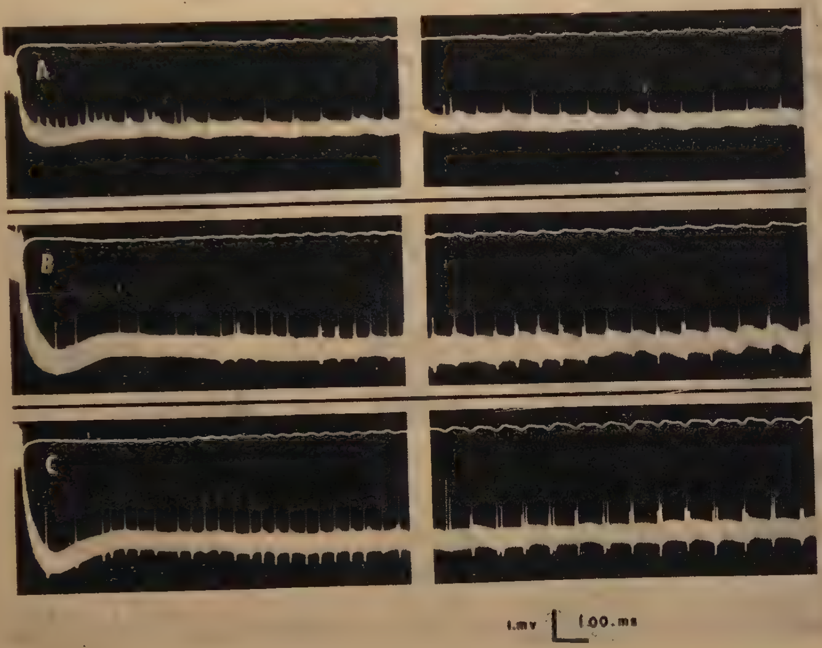

Fig. 6. – *Three examples of simultaneous recording of different units.*

Note the tendency for more units to fire in the later phases of the afterdischarges. *B*, *C*: note variability of organization in coincidence with the successive slow EEG waves.

Spike amplitude and frequency undergo a characteristic progressive change. At the very early stages of the afterdischarge the potentials recorded with the microelectrode are very low in amplitude (see Figs. 4 and 8), much smaller than in resting conditions; then their amplitude progressively increases (see same Figs.) until

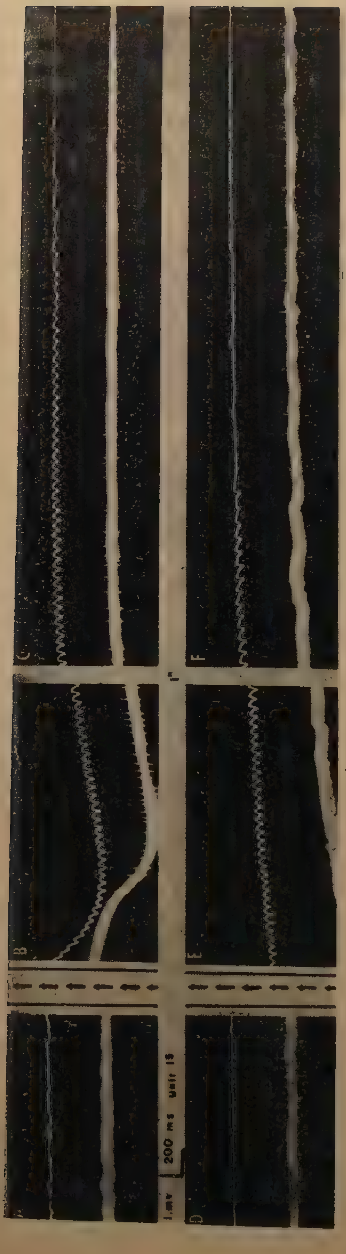

Fig. 7.

Fig. 7. – *Difference between spontaneously active and "quiet" units.*

A, *D*: before stimulation (arro 4). *B*, *C*; *E*, *F*: during afterdischarge

Fig. 8. – *Spike frequency and amplitude evolution during afterdischarge.*

Unit 79 (*A-I*): latent period (*A*, *B*); onset of tonic phase (*B*); tonic phase (*C-F*); clonic phase (*G-I*). Note change in frequency and progressive increase of spike amplitude; only slow potentials present in *A*, *B*. Size evolution is outlined on the right: From bottom up: spike size in resting condition, then during the afterdischarge. Unit 77 (*J-R*): same phenomenon with higher speed sweep; in *J* a "spontaneous" spike. In *R* repetitive activity of same unit (see text) at the end of the afterdischarge.

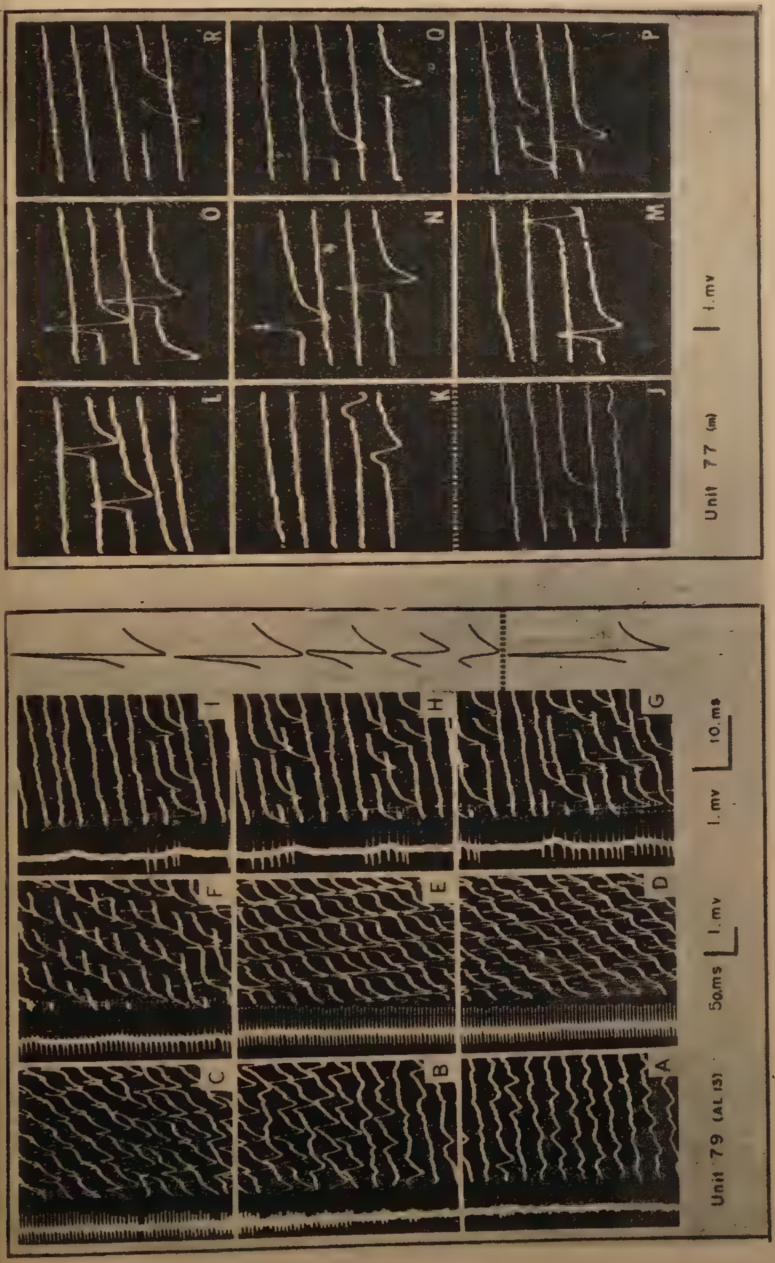
R
Q
P
O
N
M
L
K
J
Unit 77 (m)
1.mv
I
H
G
F
E
D
C
B
A
Unit 79 (AL 13)
50.ms
1.mv
1.mv
10.ms

Fig. 8.

the spike approaches the original value which is generally reached at the end of the afterdischarge. Occasionally this occurs before the spike amplitude has recovered to the original value, while on rare occasions the original amplitude may be slightly exceeded. This amplitude evolution is not so apparent in those cases in which the spike appears late in the course of the afterdischarge; in these cases it has a size which is either " normal " or very close to its original value and the subsequent changes are, therefore, hardly noticeable (see for instance Figs. 5, 6).

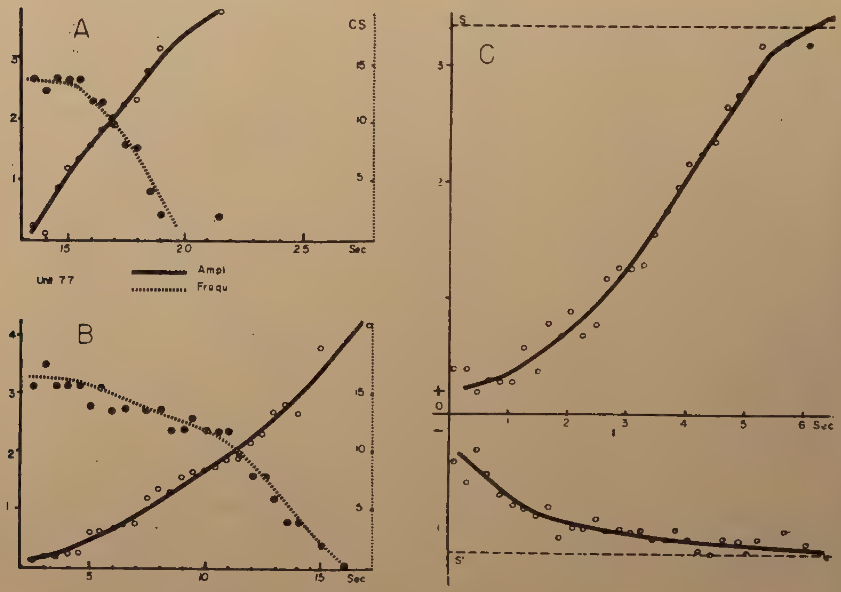

Fig. 9. – *Graphic representation of phenomena shown in Fig. 8.*

A, *B*: evolution of spike size (black line) and frequency (dotted line) during two different afterdischarges for the same unit. Frequency (c/sec) on the right, amplitude (arbitrary units) on the left ordinate. Time in sec (from the end of the stimulation). *C*: evolution of both positive and negative phases of a spike (unit 79 of Fig. 8) during afterdischarge. The dotted lines indicate the amplitude levels of these two phases for the same spike in resting conditions. These graphs were obtained by plotting the average values for amplitude and frequency for 500 msec (*A*, *B*) and 200 msec (*C*).

The course of this amplitude evolution is graphically represented in sections *A* and *B* of Fig. 9; while the selective changes in amplitude for positive and negative components of the spike are shown graph *C* of the same illustration. In this same figure we see the lotting of the evolution of the firing frequency of each spike. hroughout the afterdischarge this frequency progressively decreases an inverse relationship with the amplitude of the spikes.

The regular, rhythmical type of firing which is very common the early tonic phase often may change, in the second part of he tonic phase or during the clonic stage, in a more complex pattern.

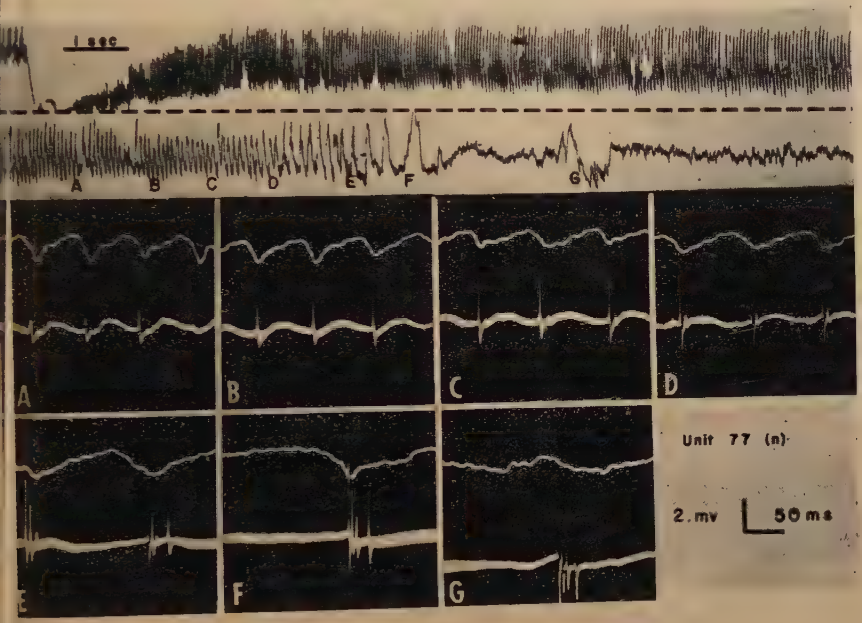

Fig. 10. – *Example of repetitive firing.*

This unit starts firing at the beginning of the clonic phase. Note progressive increase in amplitude. In *C* repetitive firing starts and this tendency increases until in *E*, *F*, *G*, typical clonic bursts are visible.

This phenomenon is illustrated in Fig. 10 where, in coincidence with each slow wave of the EEG discharge, a spike begins to fire 2 then 3 times and eventually with a more and more repetitive character. As already noted when presenting the results during electrical stimulation, in these bursts the size of the various spikes is not uniform, and generally the 2nd and 3rd spikes are smaller than the first, there being a direct relationship between their amplitude and the duration of the interval which separates them from the preceding one (Figs. 2 *A*, *C*, 10 *D-G*, 11). Generally the spike frequency in these bursts does not exceed 200/sec. Such a phenomenon is particularly characteristic in correspondence with the " clonic bursts " often observed in the EEG tracing toward the end of an afterdischarge or constituting the predominant pattern in the so-called " larval afterdischarges " in those cases in which the electrical stimulation was barely adequate or it followed too closely a previous, fully developed afterdischarge.

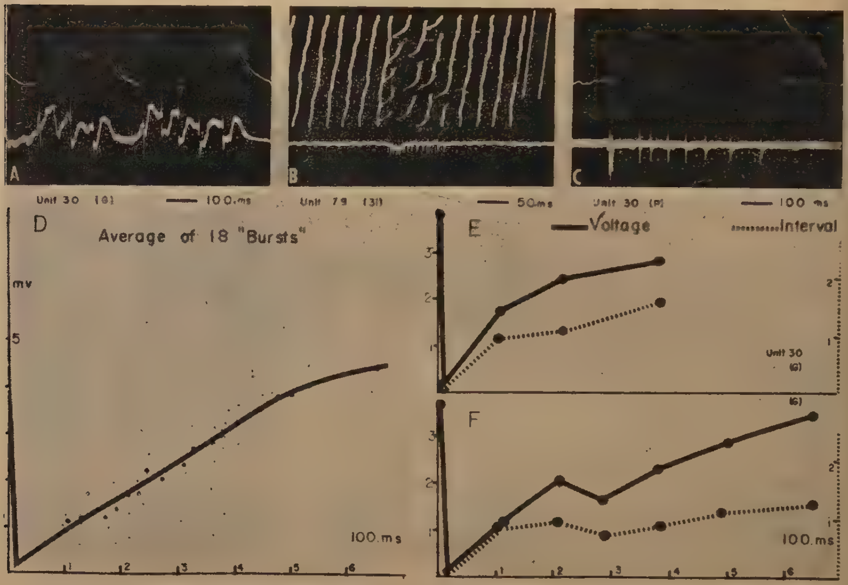

Fig. 11. – *Clonic bursts.*

A, B, C: 3 examples for 2 different units. *D*: spike amplitude evolution in 18 " bursts " from the same afterdischarge. The small dots indicate the individual values and the circles their average in each 25 msec interval. *E, F*: relation between spike amplitude and inter-spike interval for two " bursts " (amplitude in abitrary units on the ordinate on the left).

The end of the afterdischarge is generally sudden and, as a rule, leaves place to a period of inactivity in the EEG. Most of the unit firing also stops simultaneously with the end of the surface activity and, as mentioned, when their spike amplitude has reached the resting levels. While it is possible to observe some of the units stopping their activity slightly earlier than the visible EEG discharge, practically none were seen to persist firing afterwards. This silent period not only corresponds to the arrest of the activated discharges

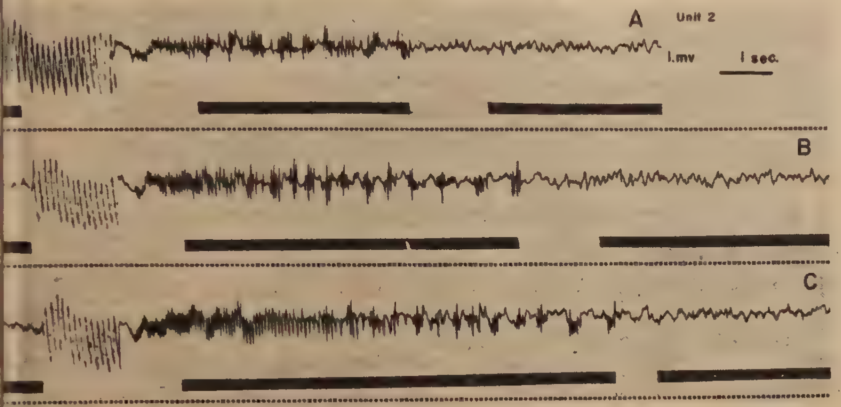

Fig. 12. – *Post-afterdischarge inhibition.*

The heavy black line indicates unit firing (see text).

but also to an actual inhibition of the spontaneous firing as it can be appreciated in those units already displaying repetitive firing in resting conditions (see Fig. 12).

DISCUSSION

A reliable interpretation of any spike change is contingent upon the assumption that the spikes under consideration express the activity of a single unit. With extracellular electrodes, simultaneous firing of different elements or, respectively, impulses from a single cell may be recorded. The accepted criterion for considering a spike as originating from a single unit is its constancy in amplitude and shape. In many of our records, spike size and shape remain quite uniform in resting conditions, yet, during electrical stimulation and the following afterdischarge, the same spikes undergo progressive decrease, disappearance and then progressive increase of amplitude until the original size is again reached. These changes, occurring in every instance of adequate stimulation and fully developed afterdischarge, could hardly express successive activations and inhibitions of different cells: their smoothly progressive character, their relation to frequency of firing and the possibility of reproducing up to 20 times, with superimposable features, the whole sequence of events, justifies the assumption of a single unit origin. We may thus generalize and safely state that definite changes in both amplitude and frequency characterize the unitary spikes during and following efficient repetitive stimulation. It is important to consider simultaneously these two types of changes since we know, from intracellular *DC* recordings, that a relation exists between polarization values on the one hand, and rate of firing and spike amplitude on the other. Minimal depolarization levels compatible with firing of an impulse are accompanied by spikes of minimum frequency and maximum size, while this relation is reversed for high depolarization levels: when these exceed a certain value, spikes are abolished (12, 16, 18).

In the light of these data it is possible to offer a hypothesis on the effects of cortical repetitive stimulation.

We may assume that a stimulus determines some depolarization of the cell membrane, the firing level being reached if the stimulating pulse is strong enough. If the stimulus is isolated or is separated from the following one by a relatively long interval (over 100 msec), the resting polarization level is then resumed, each stimulus having the same effect as the preceding one. If the stimuli are strong enough to produce a long-lasting depolarization, brief repetitive firing takes place.

With a higher stimulation frequency, complete recovery of the resting polarization level cannot occur: each subsequent stimulus falls at a level lower than the previous one and, by successive steps, a progressively higher depolarization is built. In these cases the actual spike amplitude decreases until, with sufficiently long stimulation, an excess of depolarization is reached where firing is no longer possible.

This course of events is illustrated in Fig. 13 where, for each spike, the difference in amplitude from its resting value, is plotted against time, to obtain a rough outline of the polarization levels.

At the end of the electrical stimulation, an inverse repolarization process begins. The initial silent phase of the afterdischarge corresponds to the time necessary to reach the upper limits of depolarization at which firing becomes again possible. The high frequency low amplitude spikes appear and progressively, as the resting potential is approached, the frequency decreases and the amplitude increases until the firing stops when this level is reached. These phenomena are illustrated in Fig. 13 *B*. Whether this process ends at this stage or whether it actually goes on and the cell mambrane ecomes hyperpolarized, cannot be stated. The existence of a period f cortical hypoexcitability, when the EEG is "flat", and the xamples of arrest of firing in spontaneously active units (see Fig. 12) ould suggest that the post-afterdischarge "exhaustion" (13) be n reality the expression of an inhibitory process very probably ased on hyperpolarization.

This interpretation assumes the existence of an extremely long epolarization time for the cell membrane with values of 15-100 msec n the stimulation period and up to 60 sec during the afterdischarge. We discard as unlikely the possibility of a membrane time constant of such values being actually characteristic of cortical neurones; a depolarizating after-effect following each spike could be invoked as a factor delaying the repolarization process (23), and the occurrence of biochemical changes of a slowly reversible type should also be taken into consideration. It seems, however, more probable that during and following stimulation, a considerable impingement of impulses from nearby activated neurones takes place. This steady bombardment could result in a summation of elementary post-synaptic potentials and consequently in a substantial slowing down of the repolarization process.

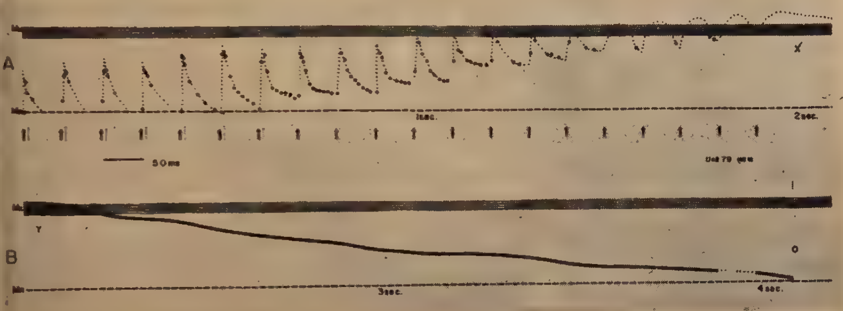

Fig. 13. – *Hypothetical course of membrane polarization.*

A: during electrical stimulation (only one every other stimulus is shown). *B*: during afterdischarge. Mn: minimum depolarization level at which firing appears; Mx: level of depolarization at which "inactivation" occurs; X: "silent" phase after the end of stimulation; O: end of afterdischarge. (See text for details).

Regardless of the actual mechanisms involved, this slow, long-lasting process can be considered the characteristic feature of the "epileptic" neuronal pool.

In our experimental conditions it is not possible to satisfactorily explain the mechanism underlying the " clonic bursts " which, as described, are characterized by repetitive firing with typical and reproducible variations in the frequency and in the amplitude of each spike. It is of interest to note that the firing pattern in these bursts is rather similar to that observed in the repetitive firing which occurs in the course of electrical stimulation. If this analogy is not purely formal, the clonic bursts seen toward the end of the afterdischarge or in cases of larval afterdischarges, could de interpreted as the expression of oscillations in the descending slope of the polarization curve. These oscillations would seem to become more appreciable in proximity to the resting polarization level. Worth noting is the similarity between this phenomenon and those described during *DC* stimulation of peripheral nerves (14) and in the spinal cord toward the end of the strychnine tetanus (7).

In regard to the end of the afterdischarge, an explanation can be offered on the basis of the above-mentioned mechanism. It is reasonable to think that the " dropping off " of a small percentage of cells is sufficient to markedly upset the " braking " mechanism which their impingements were supporting and the large majority of units will then reach, in a matter of a few milliseconds, the resting potential and stop their firing. It is also possible that an active inhibitory process, which was previously hidden or overcome by the more prominent excitatory ones, may at this time exert its effects, and be responsible for the phase of depression (hyperpolarization?), which, as a rule follows every afterdischarge.

Finally, a parallel study may be drawn between unit behavior and the well known EEG pattern of the afterdischarge. The initial latent phase corresponds to a period in which there is no firing or in which only a few, non synchronized, units are active. The onset of the afterdischarge, as appreciable in the surface record, roughly corresponds to the onset of firing of many cells, which rapidly become synchronized with a rhythm strictly close to that of the EEG. The subsequent events of the EEG afterdischarge would correspond to a progressive decrease in frequency and to an increase of number of active units parallel to a progressive increase of the actual amplitude of each spike, with, in addition, a tendency toward more and more repetitive firing for each unit with three or more spikes firing in coincidence with each slow wave. The abrupt end of the discharge corresponds to the sudden arrest of practically all the units, and the postdischarge " flat " period is not only the expression of " exhaustion " but, very likely, a true phase of inhibition.

SUMMARY

A unit analysis – performed with tungsten microelectrodes within the suprasylvian area of the cat – with the purpose of investigating the mechanism at the basis of self-sustained activity has provided the following results:

1) Repetitive stimulation, capable of eliciting afterdischarge, is accompanied by progressively developing and characteristic changes in the spikes activated by each electric pulse. Units tend to fire repetitively and the amplitude of the successive spikes decreases until they eventually might disappear.

2) In the absence of such changes the development of an afterdischarge is highly improbable.

3) During the EEG afterdischarge unit spikes, generally absent at the very beginning, progressively appear: their voltage is at first low and their frequency high, then the rate of firing slows down and their amplitude reaches the pre-stimulation value, at which point the afterdischarge ends.

These spike changes have been interpreted as the expression of different membrane polarization levels, the characteristic ones consisting of an excess of depolarization and of long lasting repolarization process.

ACKNOWLEDGEMENT

The author is duply indebted to Dr. C. Ajmone-Marsan whose permanent guidance and support made this work possible, and to Dr. M. G. F. Fuortes for his essential help and advices.

BIBLIOGRAPHY

1. ADRIAN, E. D. The spread of activity in the cerebral cortex. *J. Physiol.*, *88*: 127-161, 1936.
2. ADRIAN, E. D. and MORUZZI, G. Impulses in the pyramidal tract. *J. Physiol.*, *97*: 153-199, 1939.
3. BAK, A. F. A unit gain Cathode Follower. *EEG clin. Neurophysiol.*, *10*: 745-748, 1958.
4. BAUMGARTNER, G. Microelectrode recordings from single cortical neurons in the normal state and during epileptic discharges. *EEG clin. Neurophysiol.*, *6*: 520-521, 1954.

5. Baumgartner, G. and Jung, R. Hemmungsphänomene an einzelnen corticalen Neuronen und ihre Bedeutung fur die Bremsung convulsiven Entladungen. *Arch. Sci. biol.*, *39*: 474-486, 1955.
6. Bonnet, V. and Bremer, F. Analyse des modifications préconvulsives de la réaction de l'écorce cérébrale à un stimulus direct répété. *J. Physiologie*, *48*: 399-403, 1956.
7. Bremer, F. Le tetanos strychnique et le mécanisme de la synchronisation neuronique. *Arch. int. Physiol.*, *51*: 211-260, 1941.
8. Bremer, F. Du mécanisme de l'épilepsie faradique. *Acta neurol. belg.*, *58*: 5-9, 1958.
9. Burns, B. D. Intracortical integration. *EEG clin. Neurophysiol.*, suppl. IV, 72-81, 1953.
10. Burns, B. D. The production of after-bursts in isolated unanesthetized cerebral cortex. *J. Physiol.*, *125*: 427-446, 1954.
11. Creutzfeldt, O., Baumgartner, G. and Schoen, L. Reaktionen einzelner Neurone des senso-motorischen Cortex nach elektrischen Reizen. *Arch. Psychiat. u. Z. ges. Neurol.*, *194*: 597-619, 1956.
12. Curtis, D. R. and Watkins, J. C. Investigations upon the possible synaptic transmitter function of GABA (gamma aminobutyric acid) and naturally occurring amino acids. *Conference on inhibition in the nervous system and GABA*, Duarte, California, May, 1959.
13. Dusser de Barenne, J. G. and McCulloch, W. S. Factors for facilitation and extinction in the central nervous system. *J. Neurophysiol.*, *2*: 319-355, 1939.
14. Erlanger, J. and Gasser, H. S. *Electrical signs of nervous activity.* Univ. of Pennsylvania Press, 1936, x-221 pp. See pp. 102-110.
15. Green, J. D. and Naquet, R. Étude de la propagation locale et à distance des démarches épileptiques. *IV int. Congress of EEG clin. Neurophysiol.* Brussels, July, 1957, 225-249.
16. Granit, R. and Phillips, C. G. Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. *J. Physiol.*, *133*: 520-547, 1953.
17. Forbes, A. The interpretation of spinal reflexes in terms of present knowledge of nerve conduction. *Physiol. Rev.*, *2*: 361-414, 1922.
18. Fuortes, M. G. F. Electric activity of cells in the eye of limulus. *Amer. J. Ophthal.*, *4*: 210-223, 1958.
19. Hubel, D. H. Tungsten Microelectrode for recording from single units. *Science*, *125*: 549-550, 1957.
20. Jung, R. Origine e propagazione di potenziali convulsivi cerebrali nelle ricerche sperimentali negli animali. *Riv. Neurol.*, *21*: 333-347, 1951.
21. Jung, R., Creutzfeldt, O. and Baumgartner, G. Microphysiologie des neurones corticaux: processus de coordination et d'inhibition du cortex optique et moteur. *Microphysiologie des elements excitables.* Edit. de CNRS, Paris, *67*: 411-431, 1955.
22. Jung, R., Creutzfeldt, O. and Grusser, O. J. The microphysiology of cortical neurons. Its significance for sensory and cerebral functions. *German Medical Monthly*, *3*: 269-276, 1958.
23. Kandel, E. R., Spencer, W. A., Brinley, F. J. and Marshall, W. H. Properties of hippocampal neurones as studied by intracellular recordings. *Fed. Proc.*, *18*: 78, 1959.
24. King, R. B., Schricker, J. L. and O'Leary, J. L. Experimental study of the transition from normal to convulsoid cortical activity. *J. Neurophysiol.*, *16*: 286-298, 1953.
25. Liberson, W. T., Ellen, P. and Smith, R. W. Observations on "dendritic potentials" and seizure patterns in the hippocampus of guinea pigs. *EEG clin. Neurophysiol.*, *10*: 188-189, 1958.
26. Rosenblueth, A. and Cannon, W. B. Cortical responses to electric stimulation. *Amer. J. Physiol.*, *135*: 690-741, 1942.

Arch. ital. Biol., *98*: 21-40, 1960.

CHOLINESTERASES DISTRIBUTION IN CEREBELLUM

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INTRODUCTION

Acetylcholine (2, 17, 10) and related enzymes – true cholinesterase (1, 3, 19) and choline-acetylase (12) –, as well as catecholamines – adrenaline and noradrenaline (21) –, have been studied in a great number of sites in the brain and spinal cord. The mapping out of results, concerning loci quite different in structure and functional significance, has led to the suggestion of an alternative pattern of chemical transmission (cholinergic and non-cholinergic) in the C. N. S. (11, 9, 8).

Among different parts of C. N. S., the cerebellum has received relatively less attention. A high cholinesterase activity, found in the cerebellum by Nachmansohn (18) and Ord and Thompson (19), was shown to be predominantly localized in cerebellar cortex by Burgen and Chipman (3). Low choline-acetylase activity and acetylcholine content in the cerebellum have been reported by a number of authors (11, 13, 2, 17, 10), choline-acetylase reaching its lowest value in cerebellar cortex (11, 13). This finding fails to support the contention that the very high cholinesterase activity of the cerebellar cortex – one of the highest in the C. N. S. – may be predominantly related to a cholinergic mechanism of intracortical neurons.

The morphological and functional features of cerebellar structure (homogeneous distribution and density of cortical population, differential spreading of afferents from different sources, etc.), seem adequate for a reexamination of the problem. Data on cholinesterase activity of earlier authors (18, 19) refer to the cerebellum in toto, while those of Burgen and Chipman (3) concern only four different cortical fields (lateral lobes, flocculus, vermis and anterior lobe, the highest value being found in the latter), and one only of the central nuclei (dentate). The present paper is an attempt to give a detailed map of both ChEI (true cholinesterase) and ChEII (pseudo-cholinesterase) distribution in the cerebellar cortex, nuclei and peduncles.

Methods

Experiments were carried out on horse cerebellum. A total of 32 animals were used, ages ranging between 3 and 6 months. Cerebella, removed as soon as possible after the shooting of the animals, were placed in a dish containing ice-cold Ringer solution and brought to the laboratory, the whole operation taking not more than 20 minutes. The dissection of cortical areas was then immediately carried out. After removal of gross meninges and chorioid plexuses, special care was taken for the exact identification of different fissures and lobules. All the folia of each anatomical unit were dissected in their more distal part, freed from pial sheet and axial white matter (without removing the deeper grey layer), carefully dried on filter-paper, and finally weighed.

Nuclear samples were obtained by dividing the cerebellum frontally in two halves, then separating it in large sagittal slices. The samples were completely freed from paranuclear white matter, using a dissection microscope.

Peduncles samples were obtained from segments of their free portion.

Estimations were carried out by the Warburg manometric method. Tissue samples were first ground in a chilled mortar with fine sand, and then homogenized in a cold bicarbonate medium (0.125 M NaCl, 0.0025 M KCl, 0.018 M $CaCl_2$, 0.025 M $NaHCO_3$). The amount of medium used per gram of tissue was – according to the concentration of extracts required – 85 ml for cortical areas, 50 ml for nuclei, 30 ml for peduncles. After centrifugation, the supernatant solutions were used as enzyme preparations.

The Warburg vessels contained 1.7 ml of the enzyme solution in the main compartment and 0.3 ml of the substrate solution in the side bulb. After equilibrating with a gas mixture of N_2 95%-CO_2 5% (giving a final pH of 7.4), and attaining temperature equilibrium (37° C), the contents of the vessels were mixed and CO_2 evolution followed for 30-60 minutes according to maximal rate. Duplicate runs were always carried out. Corrections were performed for gas evolution due to spontaneous hydrolysis of substrates; no CO_2 evolution was observed in checking the tissue without substrate.

Results are expressed as μl CO_2 evolved per gram wet weight tissue in 10 minutes.

Final substrate concentrations were: acetylcholine chloride (ACh) 0.0127 M, acetyl-β-methylcholine chloride (MeCh) 0.03 M, and butyrylcholine jodide (BuCh) 0.01 M. The use of acetyl-β-methylcholine and butyrylcholine as "specific" substrates, proved in our case satisfactory to portray the different distribution of ChEI and ChEII in cerebellar cortex and in subcortical structures.

RESULTS

As far as cerebellar cortex is concerned, all 14 areas of Larsell subdivisions, as mapped out by us for horse cerebellum in Figs. 1 and 2, were taken into consideration. In the anterior lobe, a further subdivision was suggested by the different anatomical and functional properties of vermal and non-vermal (lateral and/or intermediate) strips of cortex. This subdivision was carried out in the case of l. centralis and culmen, the hemispheric portion of the lingula (if present in horse cerebellum) being too small to permit separate analysis. The same subdivision into a lateral and vermal portion was applied to l. simplex.

In examining cholinesterase activity of these seventeen cortical fields, large individual differences were found among different animals. Variability however involved absolute rather than relative figures, as shown in Table I, where the results (Q ACh)[1] of 18 different experiments are stated in absolute values only for vermian culmen, tested in each experiment, and as a percentage of this value for all other cortical fields. The last column gives the means of these percentages.

Comparative evaluation has been similarly introduced in studying Q MeCh and Q BuCh[1], to minimize the effects of variability due to separate testing of different fields. The means as reported in Table II are then more significant than would be the means of absolute values irrespective of comparative experiments.

Q MeCh/Q BuCh ratios and the percentages of ACh hydrolysed by ChEII[2] are also given in Table II: they represent mean values of several experiments in which both Q MeCh and Q BuCh were tested together.

As far as subcortical structures are concerned, all three central nuclei – fastigial, interposite and dentate – and all three peduncles –

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[1] For meaning of Q ACh, Q MeCh and Q BuCh, see Table II.

[2] In our conditions acetyl-β-methylcholine was split by ChEI at 46% of the rate of acetylcholine, and butyrylcholine by ChEII at 217% of the rate of acetylcholine. The formula:

$$\frac{46\ \text{Q BuCh}}{2.17\ \text{Q MeCh} + 0.46\ \text{Q BuCh}}$$

gives then the percentage of total acetylcholine hydrolysed by ChEII (3).

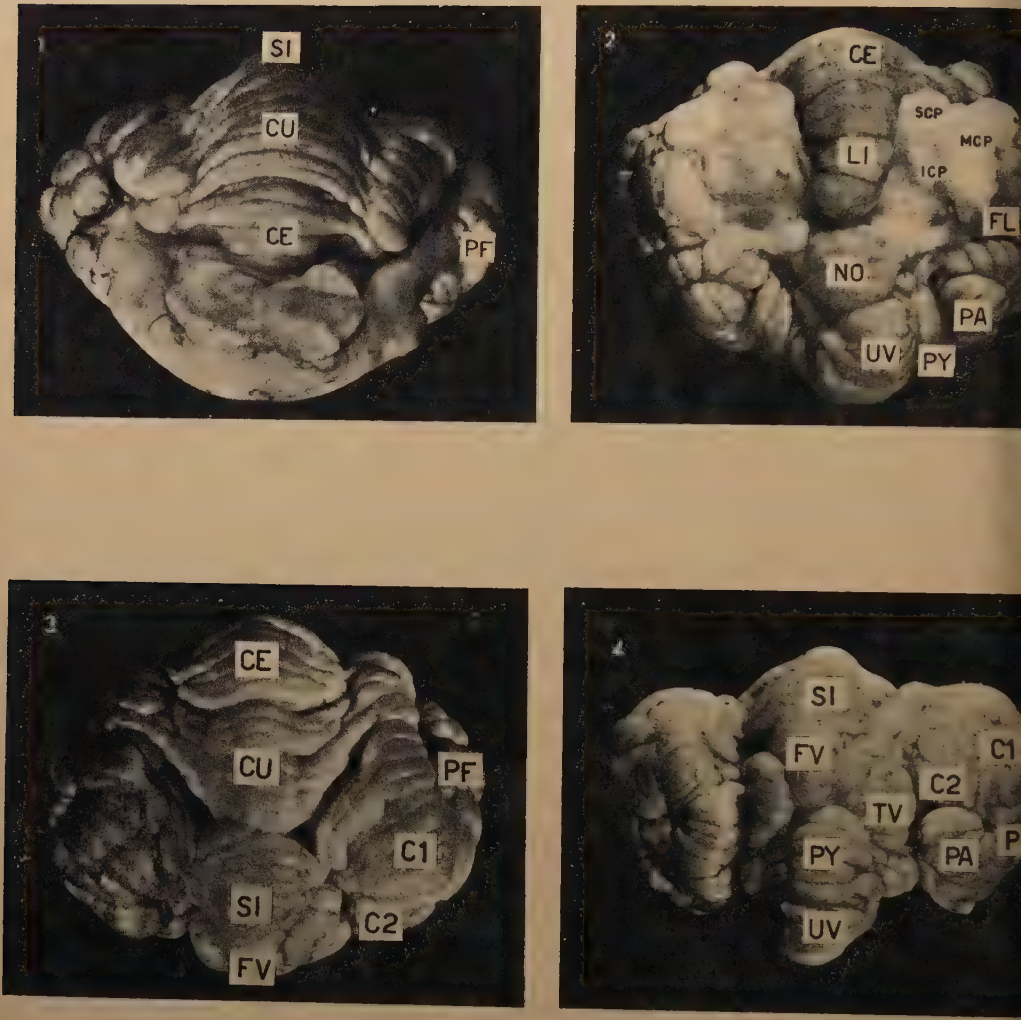

Fig. 1. – *Horse cerebellum.*

1, cranial view; 2, ventral view; 3, dorsal view; 4, caudal view. – C_1, crus primum, lobuli ansiformis; C_2, crus secundum, lobuli ansiformis; *CE*, lobulus centralis; *CU*, culmen; *D*, dentate nucleus; *F*, fastigial nucleus; *FL*, flocculus; *FV*, folium vermis; *I*, interposite nucleus; *ICP*, inferior cerebellar peduncle; *LI*, lingula; *MCP*, middle cerebellar peduncle; *NO*, nodulus; *PA*, lobulus paramedianus; *PF*, paraflocculus; *PY*, pyramis; *SCP*, superior cerebellar peduncle; *SI*, lobulus simplex; *TV*, tuber vermis; *UV*, uvula.

TABLE I. – *Distribution of cholinesterase activity in different parts of horse cerebellar cortex* [1].

Exp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Means
Culmen ver. (Q ACh [2])	3142	4067	3013	2880	2906	3271	3151	2836	2140	2885	2912	4022	5650	4080	3098	5256	3171	3890	
Lingula . . .	—	—	—	87.8	—	—	—	—	—	—	—	101.8	—	99.9	—	—	—	—	96.5
Centralis ver.	97.9	103.7	—	—	—	—	—	—	—	—	—	103.3	—	—	—	—	95.3	—	100.0
Centralis lat.	109.9	—	—	—	—	—	—	—	—		—	—	—	—	—	—	104.0	—	106.5
Culmen lat. .	91.8	—	—	—	—	—	—	—	—	99.8	—	—	—	—	—	—	112.9	—	101.5
Simplex ver. .	—	—	—	109.2	—	99.8	—	—	100.4	92.3	—	—	—	—	—	101.7	97.6	—	100.1
Simplex lat. .	—	—	—	—	—	—	—	—	112.9	—	—	—	—	—	—	—	121.0	—	116.9
Folium vermis	—	—	—	—	—	97.7	98.7	—	99.2	—	—	—	—	—	—	—	—	94.5	97.5
Tuber vermis	—	—	—	—	—	99.2	—	—	—	—	—	112.2	—	—	—	—	—	95.7	102.4
Crus I. . . .	—	—	—	—	—	—	104.0	104.5	—	—	115.2	—	—	—	104.9	101.1	100.8	—	105.1
Crus II . . .	—	—	—	—	—	—	96.0	—	—	—	—	—	—	—	105.3	92.8	—	—	98.0
Paramedianus	—	—	—	—	—	—	—	63.8	—	—	70.1	—	—	—	75.6	59.7	—	—	67.3
Pyramis . . .	79.6	82.9	—	—	—	—	—	—	—	65.9	—	—	83.2	—	—	—	—	72.8	76.9
Uvula . . .	—	—	59.2	—	—	—	—	50.6	—	—	—	69.5	—	—	—	68.0	—	55.5	60.5
Paraflocculus .	—	—	—	—	—	—	—	73.9	—	—	—	—	—	63.9	—	69.6	—	—	69.1
Nodulus . . .	—	—	90.9	—	70.1	—	—	—	—	—	—	89.1	79.2	71.4	111.3	—	96.9	110.7	91.2
Flocculus. . .	—	—	—	—	86.2	—	—	—	—	—	—	101.6	—	—	—	—	111.3	—	99.7

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[1] Activity of all cortical fields is expressed as percentage of value for vermian culmen.
[2] Q ACh = μl CO_2 evolved/g wet wt. of tissue/10 min with acetylcholine as substrate.

inferior, middle and superior – were considered. According to the limited number of different structures, no problem of separate testing arose. In Table III results are then referred both as mean absolute and as mean per cent values for all three substrates. In the same Table is given the relative amount of ACh hydrolysed in each case by ChEII.

The results indicate that the cholinesterase activity of cerebellar cortex is by far the highest among all structures studied. Moreover, its activity appears almost entirely due to ChEI. Q MeCh values closely parallel Q ACh distribution, and Q BuCh values, fairly constant throughout, are too low to affect significantly Q MeCh/Q BuCh ratios and ChEII % values. On the whole, the cerebellar cortex then appears to contain everywhere a constant enzymatic mixture, formed almost entirely by ChEI.

The enzymatic composition of the nuclei and peduncles is instead quite different, although fairly constant within each group of structures. The nuclei show the highest Q BuCh values, per cent activity of ChEII being almost equal to per cent activity of ChEI in the cortex. This applies to all three nuclei, as shown by equal per cent distribution of Q ACh and Q BuCh and by constant high ChEII % values. In the peduncles, Q MeCh is relatively higher than in the nuclei; ChEII % value is then intermediate between that of nuclei and cortex. In this group of structures, a difference in enzyme composition is also possible: both per cent distribution of Q ACh and Q BuCh, and ChEII % figures suggest a relatively higher ChEI activity of the middle peduncle.

Differences in enzyme level are present within each structural group. In cerebellar cortex (see map in Fig. 2), the highest values of cholinesterase activity appear more or less evenly distributed over a great number of fields, with a possible peak in the lateral simplex and centralis. A low value region seems clearly confined to the caudal part of the posterior lobe, with the lowest value in the uvula. The cognate lobules nodulus and lingula seem to have an intermediate position.

With regard to subcortical structures, no significant difference can be recognized between the fastigial and interposite nuclei; the dentate nucleus however shows a higher enzymatic activity. The superior peduncle is equaled by the middle peduncle for Q MeCh values, and by the inferior peduncle for Q BuCh values.

ABLE II. – *Per cent distribution of cholinesterases in different parts of horse cerebellar cortex* [1].

		Q ACh	Q MeCh	Q BuCh	Q MeCh / Q BuCh	ChEII%
Culmen ver.		3465	1510	451		
Culmen ver. . .	(23)	100.0%	100.0%	100.0%	3.35	6.0
Lingula	(4)	96.5	92.6	101.3	3.06	6.5
Centralis ver. . .	(5)	100.0	98.0	104.5	3.14	6.3
Centralis lat. . .	(4)	106.5	114.2	103.6	3.69	5.4
Culmen lat. . . .	(5)	101.5	99.6	112.2	2.97	6.7
Simplex ver. . .	(7)	100.1	102.1	101.9	3.35	6.0
Simplex lat. . .	(4)	116.9	112.4	110.5	3.41	5.8
Folium vermis .	(6)	97.5	100.7	102.9	3.28	6.1
Tuber vermis . .	(5)	102.4	99.3	101.8	3.26	6.1
Crus I	(7)	105.1	104.5	103.3	3.38	5.9
Crus II	(5)	98.0	99.6	81.9	4.07	5.0
Paramedianus .	(5)	67.3	70.9	88.5	2.68	7.3
Pyramis	(5)	76.9	75.1	95.1	2.64	7.4
Uvula	(6)	60.5	52.8	103.3	1,71	11.0
Paraflocculus . .	(5)	69.1	76.3	115.1	2.22	8.7
Nodulus.	(8)	91.2	92.8	104.3	2.98	6.6
Flocculus	(4)	99.7	99.1	110.6	3.00	6.6

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[1] Absolute values are reported for vermian culmen. Q ACh, Q MeCh, Q BuCh = μl CO_2 evolved/g wet wt. of tissue/10 min, with acetylcholine, acetyl-β-methylcholine and butyrylcholine respectively as substrates. ChEII% = percentage of total acetylcholine hydrolysed by ChEII (see footnote p. 43). Figures between brackets refer to the number of animals used in testing each area.

TABLE III. – *Distribution of cholinesterases in nuclei and peduncles* [1].

		Q ACh ±S.D.	Q MeCh ±S.D.	Q BuCh ±S.D.	ChEII%	Q ACh%	Q BuCh%
en ver. . .	(23)	3465±885	1510±231	451±134	6.0	—	—
ate N. . .	(5)	557±101	29±4	1057±193	88.5	100.0	100.0
posite N. .	(5)	311±87	30±5	617±196	81.3	55.9	58.4
igial N. . .	(5)	357±42	47±13	698±81	75.9	64.1	66.1
erior C. Ped.	(4)	230±77	42±19	319±71	61.7	100.0	100.0
lle C. Ped.	(4)	146±21	42±4	157±25	44.2	63.4	49.2
rior C. Ped.	(4)	165±69	33±5	245±69	61.2	71.8	76.8

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[1] Absolute values are reported for vermian culmen. Q ACh, Q MeCh, Q BuCh, and ChEII% are as defined in Table II. Q ACh% and Q BuCh% = per cent distribution of Q ACh and Q BuCh referred to dentate nucleus and superior cerebellar peduncle (which showed the highest group activity).

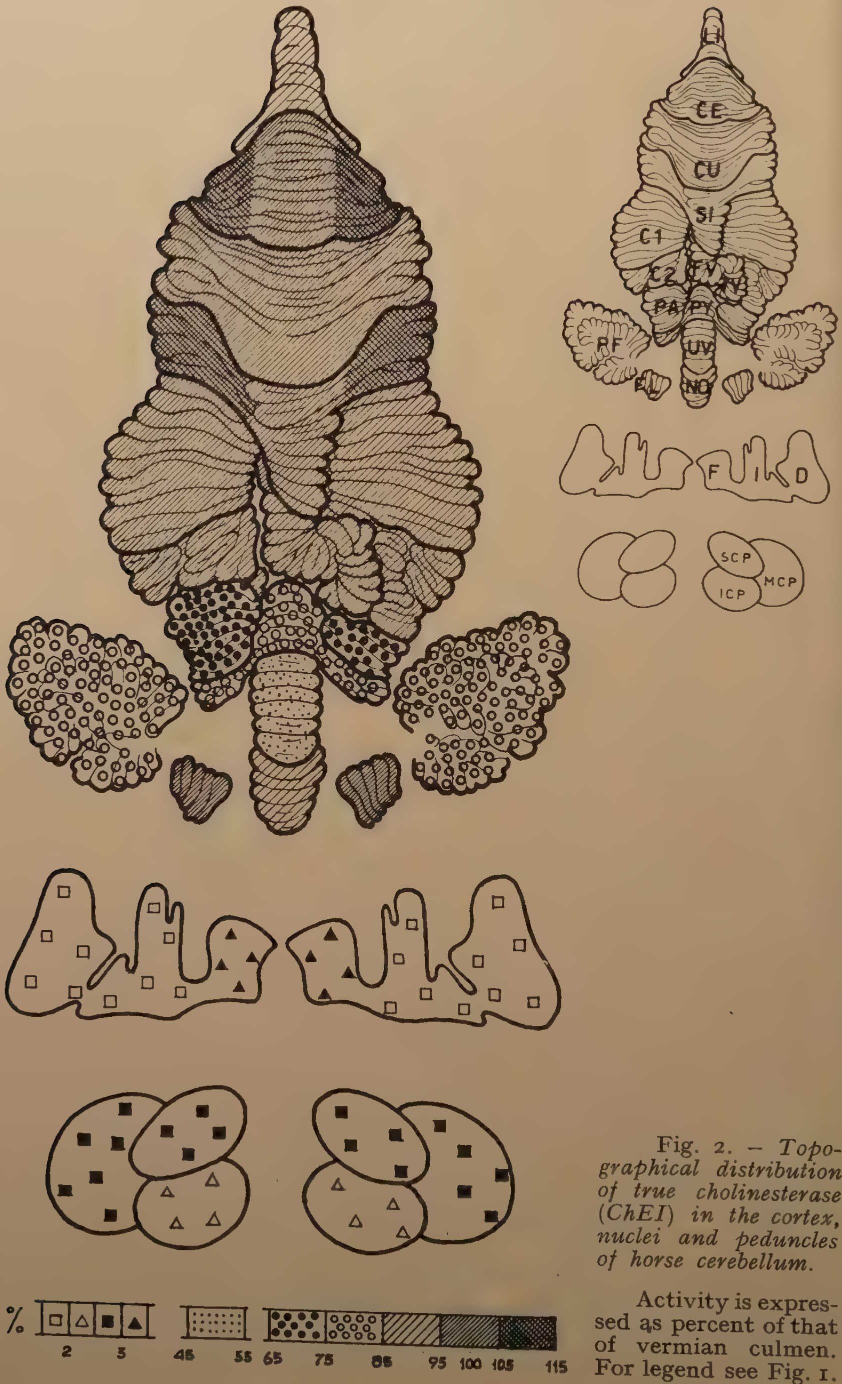

Fig. 2. – *Topographical distribution of true cholinesterase (ChEI) in the cortex, nuclei and peduncles of horse cerebellum.*

Activity is expressed as percent of that of vermian culmen. For legend see Fig. 1.

Discussion

The cerebellar cortex, nuclei, and peduncles, represent (in the horse) three morphological structures having quite different cholinesterase activity. A certain pattern of differential topographic distribution is present within each group of structures, values however never reaching beyond the typical group level. The cortical layers show by far the highest cholinesterase activity – almost entirely due to ChEI –, while the subcortical structures bring into play a lower activity, slightly more evident in nuclei than in peduncles, and predominantly due to ChEII in the former, to both ChEI and II in the latter.

These results substantially confirm findings of previous authors, stating a very high activity – one of the highest in the C. N. S. – of the cerebellum in toto (18, 19) and the cerebellar cortex (3). The latter appears to be largely the predominant site of ChEI; consequently attention is focussed on this structure for the differential topographic distribution of enzyme activity.

Although the significance of ChEI in the cerebellum still remains an unsolved problem, it is clear that it cannot be explained in terms of a cholinergic mechanism of intracortical neurons: the low acetylcholine content (2, 17, 10), and the low acetylcholine synthesizing power (11, 13) of cerebellar cortex [1], appears to be a serious drawback.

On the other hand, evidence has been given for definite cholineacetylase activity of some tracts (spino-cerebellar, ponto-cerebellar) and nuclei of origin (vestibular, posterior horn) (11, 9) of the cerebellopetal systems, as well as for a high sensitivity of cortico-cerebellar neurons to acetylcholine (5). Both facts tentatively suggest that the high ChEI activity of cerebellar cortex may be possibly linked with a cholinergic mechanism of afferent fibres [2].

This hypothesis would account for the uneven distribution of

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[1] Data for ACh refer to cerebellum in toto, but its distribution usually parallels that of the ACh synthesizing power (3).

[2] According to current views, the occurence of the three components of the ACh system (acetylcholine, true cholinesterase and cholineacetylase) is regarded as conclusive for identifying a neuron as cholinergic, i. e. as operating by the release of acetylcholine at its nerve endings. Recent evidence points out that both enzymes are derived from the cell-body and carried along the axon, ChEI being concentrated at the synaptic level (see 12). This is not always the case for choline-acetylase (20); its low value in cerebellar cortex could be due to the overwhelming mass of non-cholinergic neurons embedding some cholinergic terminals.

ChEI over the structurally uniform cerebellar cortex, since afferents from various sources (spinal, bulbar, vestibular, olivary and pontine) end in the cerebellar cortex according to a typical pattern of distribution, as far as the topography and density – electrophysiological (7) and anatomical (15) evidence – of terminals are concerned.

Unfortunately, the extensive overlapping of afferent (except vestibular) systems, and the difficulty in quantizing their relative importance, make a general analysis of our map, from this point of view, almost impossible. Some considerations might be suggested by a number of coincidences, but the only ones sufficiently substantiated to be mentioned seem to be the correspondence between the ChEI activity of the anterior and posterior (caudal part) lobes and the selectivity of their somatotopic organization; and the consistence between the ChEI activity of the flocculo-nodular cortex and the choline-acetylase activity of its private vestibular inflow.

A definite choline-acetylase activity in the cerebellar peduncles supports our hypothesis. Should there be some doubt regarding inferior and superior peduncles, which contain both afferent and efferent tracts (the latter consisting chiefly of axons from cerebellar nuclei), no problem arises when we consider the entirely afferent middle cerebellar peduncle. Feldberg's data (11) show that the acetylcholine synthesizing power is higher for both the superior and the middle peduncles; choline-acetylase distribution is paralleled by the small but definite ChEI activity, the lowest values being reached in each case in the inferior peduncle.

In all three central nuclei, ChEI activity is by no means higher than in peduncles. This seems to agree with the lack of choline-acetylase in cerebellar cortex, since both findings make it extremely improbable that the Purkinje neurons are cholinergic in nature. The choline-acetylase activity of the cerebellar nuclei [figures, intermediate between those of cortex and peduncles, refer to all three nuclei pooled together (11)] suggests that a fraction of their neurons may be cholinergic, but the extent of this cholinergic pool, and its exact location, remain a matter of speculation.

The pattern of organization of many sensory systems [1] could tentatively indicate the dentate-thalamic tract as cholinergic: a neral homology with other ascending pathways would then be alized by a peculiar arrangement consisting of the splitting of the cond neuron by a chain of non-cholinergic neurons (cerebellar rtex) outside the brain stem.

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[1] Evidence has been given by Feldberg *et al.* (11, 9) of an alternative pattern of chemical transmission often occurring in sensory pathways: the second neuron only is cholinergic, while the first order one and the last (talamo-cortical) are non-cholinergic in nature.

Finally, a few words about ChEII distribution. According to ost authors, this enzyme would be predominantly located in the glia ements (14, 4, 16), and, with few exceptions (6), has not been nown to enter in the neuronal activity of the C. N. S. The almost en distribution of ChEII over cerebellar cortex, and the presence three quite different levels of activity in cerebellum, could then ly indicate a different density of glia population in cortical and bcortical structures.

SUMMARY

1. Cholinesterase activity has been studied in horse cerebellum the Warburg manometric method, using acetyl-β-methylcholine d butyrylcholine as "specific" substrates of ChEI and ChEII spectively. Seventeen cortical fields, the fastigial, interposite and ntate nuclei, and all three peduncles have been examined.

2. The cerebellar cortex shows the highest cholinesterase acti-ty, almost entirely due to ChEI. Less activity is noticeable in bcortical structures; there is a predominance of ChEII in the clei, while in the peduncles both ChEI and ChEII contribute to most the same extent.

3. Differential patterns of enzyme distribution have been found all three groups of structures, and have been mapped out.

4. The results have been discussed on the base of present know-ge on chemical transmitters. The hypothesis of a cholinergic ture of cerebello-petal systems and, possibly, of some cerebello-gal ones (dentate-thalamic) gives us a picture of this peculiar cending system which is in close agreement with the general pat-n of organization of many sensory pathways.

ACKNOWLEDGEMENT

We are very grateful to Professor G. Stella for his advice and criticism.

REFERENCES

AUGUSTINSSON, K.-B. Cholinesterases. A study in comparative enzymology. *Acta physiol. scand.*, *15*, suppl. *52*: 1-182, 1948.

BARSOUM, G. S. The acetylcholine equivalent of nervous tissue. *J. Physiol.*, *84*: 259-262, 1935.

3. Burgen, A. S. V. and Chipman, L. M. Cholinesterase and succinic-dehydrogenase in the central nervous system of the dog. *J. Physiol.*, *114*: 296-305, 1951.
4. Cavanagh, J. B., Thompson, R. H. S. and Webster, G. R. The localization of pseudocholinesterase activity in nervous tissue. *Quart. J. exp. Physiol.*, *39*: 185-197, 1954.
5. Crossland, J. and Mitchell, J. F. The action of brain extracts, acetylcholine and histamine on the electrical activity of the cerebellum. *J. Physiol.*, *129*: 19-20 P, 1955.
6. Desmedt, J. E. and La Grutta, G. The effect of selective inhibition of pseudocholinesterase on the spontaneous and evoked activity of the cat's cerebral cortex. *J. Physiol.*, *136*: 20-40, 1957.
7. Dow, R. S. and Moruzzi, G. *The physiology and the pathology of the cerebellum.* Minneapolis, The University of Minnesota Press, 1958, VII-675 pp. See pp. 158-253.
8. Feldberg, W. S. Transmission in the central nervous system and sensory transmission. *Pharmacol. Rev.*, *6*: 85-93, 1954.
9. Feldberg, W., Harris, G. W. and Lin, R. C. Y. Observations on the presence of cholinergic and non-cholinergic neurons in the central nervous system. *J. Physiol.*, *112*: 400-404, 1951.
10. Feldberg, W. and Mann, T. Properties and distribution of the enzyme system which synthesizes acetylcholine in nervous tissue. *J. Physiol.*, *104*: 411-425, 1946.
11) Feldberg, W. and Vogt, M. Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol.*, *107*: 372-381, 1948.
12) Hebb, C. O. Biochemical evidence for the neural function of acetylcholine. *Physiol. Rev.*, *37*: 196-220, 1957.
13. Hebb, C. O. and Silver, A. Choline acetylase in the central nervous system of man and some other mammals. *J. Physiol.*, *134*: 718-728, 1956.
14. Hebb, C. O., Silver, A., Swan, A. A. B. and Walsh, E. G. A histochemical study of cholinesterases of rabbit retina and optic nerve. *Quart. J. exp. Physiol.*, *38*: 185-191, 1953.
15. Jansen, J. and Brodal, A. *Aspects of cerebellar anatomy.* Oslo, J. Grundt Tanum, 1954, 423 pp. See pp. 83-188.
16. Koelle, G. B. and Wagner, H. H. The histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. *J. Pharmacol.*, *114*: 167-184, 1955.
17. MacIntosh, F. C. The distribution of acetylcholine in the peripheral and the central nervous system. *J. Physiol.*, *99*: 436-442, 1941.
18. Nachmansohn, D. Cholinestérase dans le système nerveux central. *Bull. Soc. Chim. biol., Paris*, *21*: 761-796, 1939.
19. Ord, M. G. and Thompson, R. M. S. Pseudo-cholinesterase activity in the central nervous system. *Biochem. J.*, *51*: 245-251, 1952.
20. Sawyer, C. H. and Hollinshead, W. H. Cholinesterases in synaptic fibres and ganglia. *J. Neurophysiol.*, *8*: 137-153, 1945.
21. Vogt, M. Sympathomimetic amines in the central nervous system. *Brit. med. Bull.*, *13*: 166-171, 1957.

DEVELOPMENT OF CHOLINESTERASE ACTIVITY IN THE CEREBELLUM AND OPTIC LOBES OF THE CHICK EMBRYO

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INTRODUCTION

The present paper concerns the comparative rate of development of cholinesterase activity in the cerebellum and optic lobes of the chick. Both true (ChEI) and pseudo- (ChEII) cholinesterase have been taken into consideration, and attention have been focussed on embryonic life in relation to the efficient control of posture and movements in the newborn individual.

Cholinesterase development in the whole brain has been studied extensively – antenatally and postnatally – both in the mammals (9, 13, 7, 5, 4, 2) and in the chick (8). Only a few works, however, concern a comparative study of development of activity in different parts of the C. N. S. (9, 13, 7, 8), and no systematic data are available for cerebellum. Those of Nachmanshon on the chick (8) refer only to the 16th day of incubation and to the first few days of postnatal life.

METHODS

Experiments were performed on chick embryos from the 12th to 19th day of incubation and on newborn chickens for a few days (1-6) after hatching. Cerebella and the dorsal part of optic lobes were removed as soon as possible after decapitation of the animals, and the corresponding parts pooled together from a sufficient number of individuals (3 to 15, according to size), so as to give in each case a suitable amount of material and reliable figures of mean weight of cerebellum.

Estimations of enzyme activity were carried out on the tissue samples homogenized in 30 times their volume of bicarbonate medium (0.125 M NaCl, 0.0025 M KCl, 0.018 M $CaCl_2$, 0.025 M $NaHCO_3$). The Warburg manometric method was used, as previously described (12). Final substrate concentrations were: acetylcholine (AcCh) 0.004 M, acetyl-β-methylcholine (MeCh)

0.03 M, and butyrylcholine (BuCh) 0.01 M. Results – corrected for non-enzymic hydrolysis of substrates – are expressed as μl. CO_2 evolved per gram wet weight of tissue (or per cerebellum) in 10 minutes. No CO_2 evolution was observed in testing the tissue samples without substrates.

RESULTS

Fig. 1 shows the absolute values and the pattern of development of cholinesterase activity[1] in the cerebellum and optic lobes of the chick during the last 10 days of incubation and the first 6 days after hatching (22-27th day from the beginning of incubation).

First of all it may be seen that both in cerebellum and optic lobes enzymatic activity is already present at the beginning of our experiments, *i. e.* in the 12-day-old embryos; while moreover acetyl-β-methylcholine is hydrolysed at a considerable rate faster in optic lobes than in cerebellum, the reverse is true for butyrylcholine, which is hydrolysed slowly and with the lowest values in optic lobes. This general pattern of different cholinesterase activity was maintained during all the period of our investigation.

During the two days immediately following the beginning of our experiments, no striking alteration occurs in either enzymatic activity of the two organs, values dropping slightly below original ones. Starting from the 14th day of incubation, the total enzymatic activity of both organs increases sharply, as from the 17th day with a constant daily increment until birth. The rate then falls off, values at hatching being over 80 % of those found in the 6-day-old chickens.

The MeCh and BuCh curves clearly indicate that this pattern of development is the consequence of striking alterations occurring in true cholinesterase activity: MeCh curves parallel the changes in total enzymatic activity, whereas butyrylcholine hydrolysis rises later (16-18th day of incubation) with a low increment, and remains constant after birth.

In Fig. 2, the enzymatic activity of whole cerebellum (derived from values per gram of tissue of Fig. 1 and weight data) is compared with the increase in weight of the organ. It is clear that the gain in weight precedes the gain in cholinesterase activity, the weight urve falling off (18th day) when the activity curves are just climbng rapidly. The drop in cholinesterase activity per gram of tissue

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[1] Although the final concentration of acetylcholine (0.004 M) is close to the optimum for true cholinesterase (1), we shall refer to AcCh hydrolysis as the expression of total cholinesterase activity irrespective of type; to acetyl-β-methylcholine and butyrylcholine hydrolysis as the measure of true and pseudo-cholinesterase respectively.

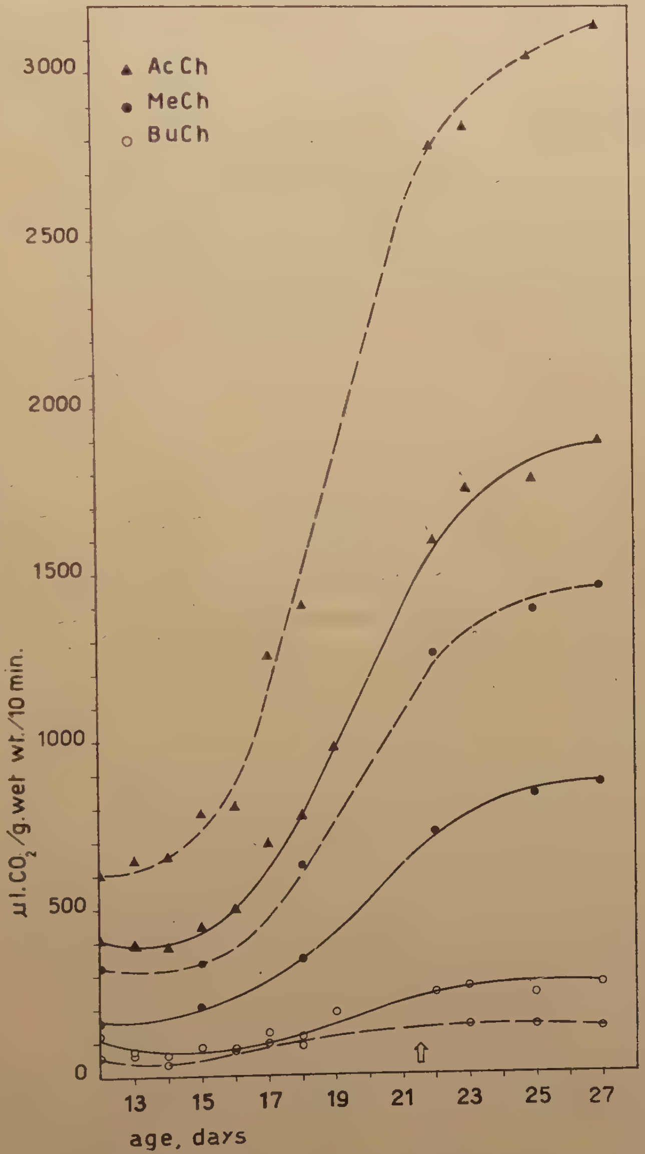

Fig. 1. – *Cho-inesterase activity f optic lobes (interupted lines) and erebellum (solid lies) in the develoing chick, before nd after hatching (indicated by the rrow).*

noted during the first few days is due to the relatively faster increase in weight of the organ, values referred to whole cerebellum rising slowly also during this period.

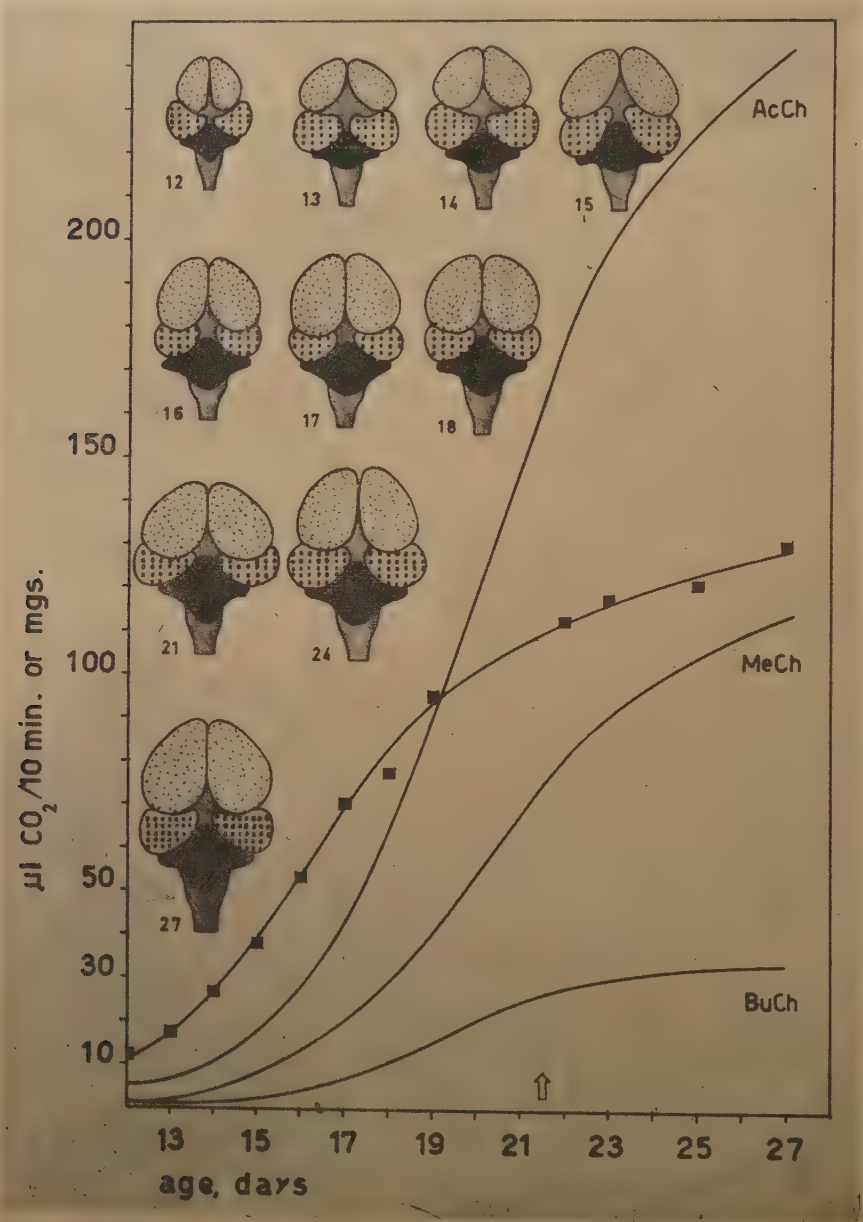

Fig. 2. – *Cerebellar weight* (■) *and cholinesterase activity per cerebellum (derived from data of Fig. 1), in the developing chick.*

Drawings outline brain development. Hatching occurs between 21st and 22nd day of incubation (see arrow).

DISCUSSION

From a general survey of these results it may be seen that although ChEI is relatively higher in the optic lobes and ChEII in the cerebellum, the general statement applies that cholinesterase activity is in each case almost entirely due to ChEI. Moreover, no significant difference can be recognized in the development of corresponding enzymes (ChEI and ChEII respectively) in the two organs.

Differences appear, on the other hand, within each structure between true hand pseudo-cholinesterase pattern of development.

Fig. 2 points out that the increase of the two enzymes in the cerebellum occurs, on a background of activity, when the organ is approaching full embryonic growth. From the weight data of Nachmansohn (8) the same idea can be reasonably applied to the optic lobes. The level and shape of the BuCh curves, however, suggest that changes in ChEII activity may be simply related to changes in volume or number, without a real gain in enzyme density, of the vascular or glial supporting elements – in which ChEII seems to be located (see 3, p. 213) – developing at a time rate slightly different from that of the whole organ.

The outstanding and critical increase in ChEI activity (simultaneous, as already stated, in cerebellum and optic lobes) can instead be apparently explained only by an increase in enzyme density in morphologically differentiated structures according to functional maturation.

Relating to the problem of the significance of ChEI in the cerebellum, it is interesting to note that in the cerebello-petal systems of the chick, myelinization (which represents the terminal phase of differentiation in nervous tissue) begins, according to Shimazono (11), – starting from the spino-cerebellar tracts – on the 12th day of incubation, and is complete at the cortical level on the 18th day. The time relationship with the development of true cholinesterase activity fits in with the previously stated hypothesis (12) that ChEI in cerebellum may be linked with a cholinergic mechanism of afferent fibres. In the absence of histochemical data concerning the chick it should be remembered that, according to Shen *et al.* (10), in the frog cerebellum true cholinesterase appears " to be largely localized in the molecular layer and the fibrous portion representing the terminals of various afferent fibres". It may be quoted that similar results were given also by Koelle (6).

Finally, the earlier development of true cholinesterase activity in the chick cerebellum as compared with that of mammals (2), can easily be accounted for when we consider that hatching produces an individual capable of controlling his posture and movements and having sufficient motor control to face independent life.

SUMMARY

True and pseudo-cholinesterase have been studied in the cerebellum and optic lobes of the developing chick embryo. ChEI accounts to a large extent for total cholinesterase activity, and develops in the two structures – although at a higher level in optic lobes – along similar curves, showing a sharp and constant daily increment starting from the 17th day of incubation. Values at hatching are over 80% of those found in the 6-day-old chickens.

ChEII develops at a much lower rate, with the same pattern in both structures, its small rise being postponed as compared with that of ChEI.

Results are discussed.

ACKNOWLEDGEMENT

We are very grateful to Professor G. Stella for encouraging this research and for his criticism.

REFERENCES

1. AUGUSTINSSON, K.-B. Assay methods for cholinesterases. In: *Methods of Biochemical Analysis*. Edited by David Glick. New York, Interscience Publishers Inc., 1957. See vol. 5, pp. 1-63.
2. ELKES J. and TODRICK A. On the development of the cholinesterases in the rat brain. In: *Biochemistry of the developing nervous system*, by Waelsch, H., New York, Acad. Press., 1955, XVII-537 pp. See pp. 309-314.
3. HEBB, C. O. Biochemical evidence for the neural function of acetylcholine. *Physiol. Rev.*, *37*: 196-220, 1957.
4. HIMWICH, H. E. and APRISON, M. H. The effect of age on cholinesterase activity of rabbit brain. In: *Biochemistry of the developing nervous system*, by Waelsch, H., New York, Acad. Press, 1955, XVII-537 pp. See pp. 301-307.
5. KAVALER, F. and KIMEL, V. M. Biochemical and physiological differentiation during morphogenesis. XV. Acetylcholinesterase activity of the motor cortex of the fetal guinea pig. *J. comp. Neurol.*, *96*: 113-119, 1952.
6. KOELLE, G. B. The histochemical localization of cholinesterases in the central nervous system of the rat. *J. comp. Neurol.*, *100*: 211-236, 1954.

7. Metzler, C. J. and Humm, D. G. The determination of cholinesterase activity in whole brains of developing rats. *Science, 113*: 382-383, 1951.
8. Nachmanshon, D. Cholinestérase dans le système nerveux central. *Bull. Soc. Chim. biol., Paris, 21*: 761-796, 1939.
9. Nachmanshon, D. Choline esterase in brain and spinal cord of sheep embryos. *J. Neurophysiol., 3*: 396-402, 1940.
10. Shen, S. C. Greenfield, P. and Boell E. J. The distribution of cholinesterase in the frog brain. *J. comp. Neurol., 102*: 717-743, 1955.
11. Shimazono, J. Das Kleinhirn der Vögel. *Arch. mikr. Anat., 80*: 397-449, 1912.
12. Sperti, L., Sperti, S. and Zatti, P. Cholinesterases distribution in cerebellum. *Arch. ital. Biol., 98*: 41-52, 1960.
13. Youngstrom, K. A. Acetylcholine esterase concentration during the development of the human fetus. *J. Neurophysiol., 4*: 473-477, 1941.

Arch. ital. Biol., 98: 53-59, 1960.

SUPRASPINAL INHIBITORY CONTROL OF TRANSMISSION TO THREE ASCENDING SPINAL PATHWAYS INFLUENCED BY THE FLEXION REFLEX AFFERENTS

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INTRODUCTION

During studies in this laboratory of the functional organization of spino-cerebellar tracts a peculiar ascending system was discovered. These neurones were activated from group II and III muscle afferents, from skin and from high threshold joint afferents (13, 17, 18, 22). The receptive field from which these neurones draw their excitation is very large, including many muscles and large areas of skin. For neurones with axons in the dorsal part of the lateral funicle the receptive field is ipsilateral, for those with more ventrally located axons the receptive field is bilateral (22). The ventral spino-cerebellar tract (VSCT) is also strongly influenced by the above system of afferents. VSCT neurones receive monosynaptic excitatory action from I *b* (Golgi tendon organ) afferents but also inhibition or and from high (and) excitation from group II and III muscle afferents, from skin, threshold joint afferents (20, 21).

As regards the possible functional significance of these ascending pathways it was at the time difficult to understand what kind of information they could convey to higher centres. Our present approach to this problem is based on the fact that the afferent systems influencing these pathways are the same that segmentally evoke the flexion reflex actions and that in both cases the receptive field is very wide (3). One possibility is that these pathways in some way may be informative with respect to the flexion reflex. It has recently been demonstrated that the interneurones transmitting flexion reflex tions from group II and III muscle afferents, from cutaneous and gh threshold joint afferents are tonically inhibited in the decebrate preparation (4, 15). The organization of this inhibitory ntrol has been investigated with respect to centres responsible and ocation in the spinal cord of descending pathways (12).

As an introduction to further studies of the ascending pathways ifluenced by the flexion reflex afferents it will be shown in this aper that in the decerebrate preparation transmission to them is nically inhibited from supraspinal centres in the same way as has een found for the flexion reflex interneurones. A preliminary acount of some of the results has been given (14).

METHODS

Most of the experiments were made on unanaesthetized cats decererated by intercollicular section. In the experiments on cats with intact rain light anaesthesia from pentobarbital sodium and pentothal was given. he activity in ascending pathways was recorded either from dissected scicles of the spinal cord or from the dissected spinal half (except the orsal column) as described in previous papers (17, 19). In a number of xperiments activity in the ascending pathways were recorded in the conentional way with steel needles electrolytically tapered and insulated except or the tip (1, 5, 6). We were able to identify the same discharges as when ecording from dissected fasciculi. However, the latter method is vastly uperior for quantitative comparisons of the present type and has therefore een used except when searching for descending controlling pathways with he same location in the cord as the ascending pathways. Preparation of egments for recording and lesions in the cord were generally performed in Th 6-10.

Condensor discharges (half time of decay 45 msec) were used for testing nd conditioning. Stimulus strengths are expressed in multiples of threshold strength for the nerve. Supramaximal is used to denote supramaximal or *A* fibres; *C* fibres were not activated by these pulses.

For reversible blockage of the descending controlling pathways the entral quadrants were dissected, the dorsal column removed as usual and thermode placed ventrally of the remaining intact dorsal part of the cord. ooled alcohol was passed through the thermode and the temperature of the ventral and dorsal side of the cord was measured with termistors. The temperature on the ventral side of cord was not allowed to fall below 0° C.

In many of the experiments Flaxedil was given and the cats held under artificial respiration. All lesions were controlled histologically as described by Laporte *et al.* (17).

All records illustrad were obtained from three superimposed traces.

RESULTS

1. *Mass discharge in dorsally and ventrally located tracts.* – In experiments with unit recording Oscarsson (22) found that among the fibres activated from high threshold muscle and from skin afferents the dorsally located had an ipsilateral, the ventral a bilateral

receptive field. Fig. 1 shows that this holds true also when recording the mass discharge. Records *A-D* were obtained from the right dissected Flechsig's fascicle. In *A* on supramaximal stimulation of the right hamstring nerve the early spikelike discharge was evoked by impulses in group I afferents and the short latency discharge in *B* on stimulation of the right sural nerve is also monosynaptically evoked (17). The later longlasting discharge in *A* is caused by activity in the pathway activated by the flexion reflex afferents (17, 18). Record *C* and *D* were obtained with identical recording on supramaximal stimulation of the corresponding contralateral nerves. In the control records *E* and *F* the same left nerves were stimulated but recording made from the right spinal half, to show the discharge

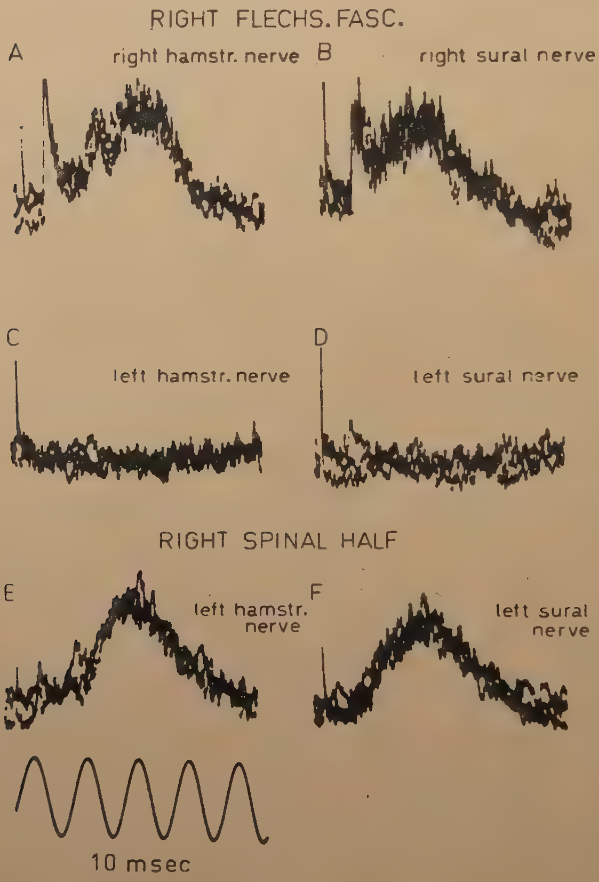

Fig. 1. – *Differentiation of mass discharge in dorsally and ventrally located pathways activated by the flexion reflex afferents.*

Recording was made from the right dissected dorsal part of the lateral funicle (Flechsig's fascicle) in *A-D* and from the right spinal half (except the dorsal column) in *E* and *F*. Supramaximal stimuli were given to the right hamstring nerve (*A*), the right sural nerve (*B*), the left hamstring nerve (*C* and *E*) and the left sural nerve (*D* and *F*). Observe absence of discharge in Flechsig's fascicle on the right side on stimulation of nerves of the left side. Spinal unanaesthetized preparation.

n the ventral pathway. Hence mass discharge recording suffices n order to distinguish between the two pathways. In the following xperiments the activity in the dorsal pathway has been recorded rom the dissected Flechsig's fascicle; for investigation of the ventral athway with bilateral inflow, recording from the spinal half and stimulation of contralateral nerves has been used or else recording rom the dissected ventral quadrant and stimulation of nerves from ither side.

2. *Comparison of effects in decerebrate and spinal animals.* — The records of Fig. 2 show the mass discharge in the dissected Flechsig's

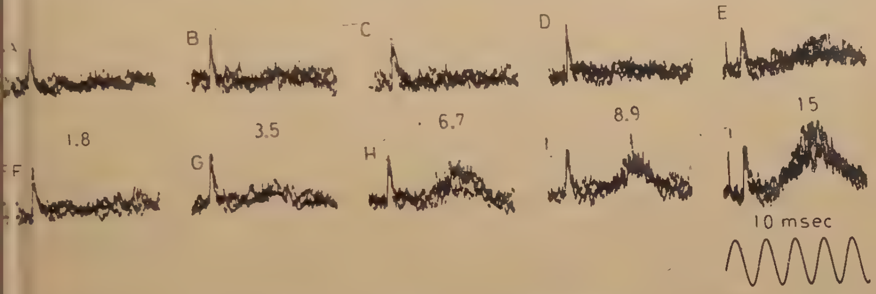

Fig. 2. – *Comparison of transmission from muscle afferents to pathways in Flechsig's fascicle in the decerebrate and spinal state.*

These records were obtained from the dissected Flechsig's fascicle on stimulation ipsilaterally at increasing strengths of the nerve to gastrocnemius-soleus. The cat was decerebrate and unanaesthetized. Upper records *A-E* were taken before, lower records (*F-J*) after section of the spinal cord. Corresponding records were obtained at the same stimulus strength, given in multiples of threshold strength. Observe large release of late mass discharge after cord section.

fasciculus on graded stimulation of the nerve to gastrocnemius-soleus. The upper records were obtained in the non-spinal state (except for the section of Flechsig's fasciculus on one side) and the lower corresponding records after section of the cord. The release of late discharge is evident, before section of cord no late discharge is found at a stimulus strength at 8.9 times threshold and only a very small discharge at 15 times threshold compared with the large late discharge in *J*. On the other hand there is no change in the monosynaptically transmitted group I effect.

From the findings in Fig. 2 it can be concluded that transmission to the dorsal of the two pathways activated by the flexions reflex afferents is strongly inhibited in the decerebrate preparation.

The records of Fig. 3 were obtained in the same experiment with stimulation of a skin nerve. As in Fig. 2 the late phase of the mass discharge is very small before section of the cord whereas the early monosynaptically transmitted discharge is unchanged. In each record in Fig. 3 the upper traces were obtained from the nerve to the knee flexors, posterior biceps-semitendinosus; the release of the flexor reflex appears from a comparison of corresponding traces.

The control of the ventral pathway is illustrated in Fig. 5 and 9. In Fig. 5 the left hamstring nerve was stimulated at two different strengths and recording was made from the right spinal half. With intact left spinal half the late mass discharge is small; it is shown at 10 times threshold for the nerve in *A* and at supramaximal stimulation in *B*. The corresponding records *E* and *F* were obtained after complete section of the cord with the appearance of the usua very large late mass discharge. The release in Fig. 9 is even more impressive. In this experiment only the dorsal half of the lateral

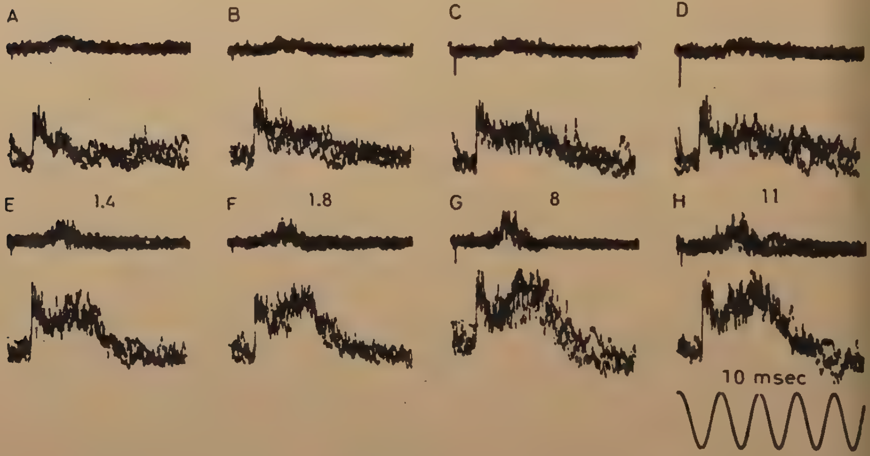

Fig. 3. – *Comparison of transmission from cutaneous afferents to pathways in Flechsig's fascicle in the decerebrate and spinal state.*

In each record the lower trace shows the mass discharge in the dissected Flechsig's fascicle and the upper trace the activity in the nerve to post. biceps-semitendinosus. Unanaesthetized decerebrate preparation. The ipsilateral sural nerve was stimlated at increasing strengths, in *A-D* before and in *E-H* after section of the spinal cord. Corresponding records were obtained at the same stimulus strengths given in multiples of threshold strength.

nicles were intact; both ventral quadrants had been sectioned d recording was made from the right dissected ventral quadrant, *A* and *D* on supramaximal stimulation of the hamstring nerve, *B* and *E* on supramaximal stimulation of the sural nerve. The rly spike in *A* is the monosynaptically transmitted I *b* volley in SCT, there is no late discharge; following blockage of conduction the intact dorsal part of the cord by cooling, the large discharge *D* is released. A volley in a skin nerve on the other hand gives me effect before blockage (*B*), but the increase afterwards is four-ld (*E*). Hence it can be concluded that transmission from the xor reflex afferents to the ventrally located tract is powerfully nd tonically inhibited from supraspinal centres in the decerebrate eparation.

High threshold muscle afferents exert a powerful inhibitory t on on VSCT neurones which can be demonstrated by conditioning e monosynaptically trasmitted I *b* discharge with volleys in high reshold muscle afferents (21). A supraspinal tonic control of me-iation of inhibition to VSCT is illustrated in Fig. 10 and 4. In ig. 10 the VSCT discharge was recorded from the right spinal half n stimulation of the left hamstring nerve. In the decerebrate state , *G*) a conditioning volley from the nerve to the left gastrocnemius-leus at 9,5 times threshold did only exert a small inhibitory effect ut after section of the left spinal half the inhibition was complete *J*, *K*). With strong conditioning volleys comprising most of group III marked inhibition was usually found also before transection of e remaining spinal half. However, it was always possible to de-onstrate the existence of a supraspinal inhibitory control to the terneurones mediating inhibition to VSCT neurones with the usage f submaximal conditioning volleys. The curves in Fig. 4 were ob-ained in a preparation in which the ventral quadrants had been ectioned, the dorsolateral quadrants remaining intact. The testing SCT discharge was evoked by synchronized volleys from the nerves o post. biceps-semitendinosus and gastrocnemius-soleus and recorded rom the contralateral ventral quadrant. In this case there was a ery effective control of the inhibitory pathway to the VSCT neu-ones; the conditioning volley (about 36 times threshold) from the erve to flexor digitorum longus gives only a small inibition in the pper curve. Similarly, in the lower curve, a conditioning volley from skin nerve has only a small inhibitory effect, compared with the arge effect found in the spinal state.

From this experiments it can be concluded that the interneurones mediating inhibitory action to VSCT neurones in the decerebrate preparation is subject to a tonic inhibition from supraspinal centres. There was no evidence suggesting a tonic inhibitory control of the VSCT neurones. Even when tested from large muscle nerves the VSCT neurone pool often displays an appreciable subliminal fringe for I *b* excitation (21). The subliminal fringe was never found to be larger in the decerebrate state, as presumably would have been the case if these neurones were tonically inhibited in the decerebrate state.

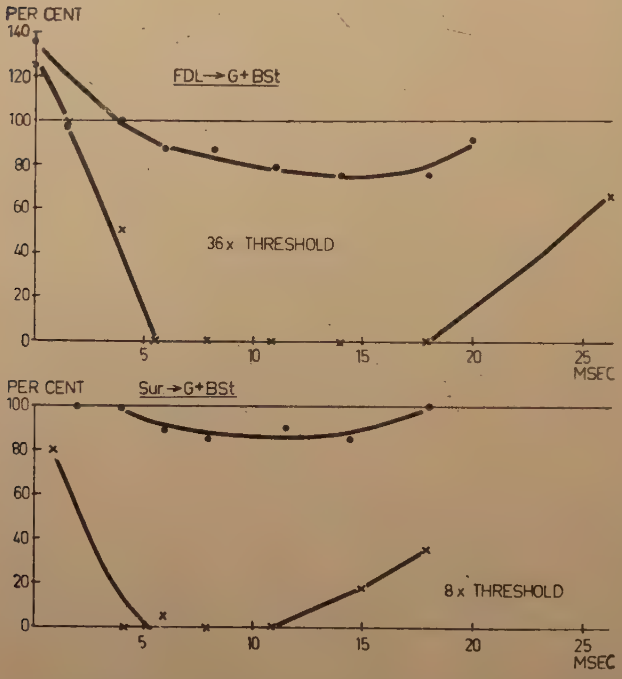

Fig. 4. – *Demonstration of tonic suppression from higher centres of inhibitory actions exerted by somatic afferents on neurones of the ventral spinocerebellar tract.*

To obtain these curves the test VSCT discharge recorded from the right dissected ventral quadrant of the cord on combined stimulation of the nerves to gastrocnemius-soleus and post. biceps-semitendinous on the left side was conditioned by volleys from the left flexor digitorum longus nerve (upper curve) and the left sural nerve (lower curve). In each instance 100% on the ordinates represents the unconditioned amplitude of the test VSCT discharge. Conditioned amplitude of the test discharge, expressed as percentage of control amplitude is plotted as a function of time interval between incoming conditioning and testing volleys. The initial facilitation by the conditioning volley in the upper curve was evoked at group I strength and is due to convergence of monosynaptic excitatory action by I *b* impulses from flexor digitorum longus and the nerves used for testing.

The experiment was made on a decerebrate cat in which both ventral quadrants had been sectioned and the effects were examined before (●) and after (×) cold blockage of the dorsal cord.

For each nerve the strength of the conditioning stimulus is expressed as multiples of threshold strength.

3. *Location of descending spinal pathways responsible for the tonic inhibitory control.* — It is known that the spinal pathways responsible for the tonic inhibitory control of flexion reflex interneurones are located in the dorsal part of the lateral funicles and that from each side a bilateral effect is exerted (12). It will presently be demonstrated that this holds true also for the pathways controlling transmission of excitation to the two ascending tracts activated by the flexion reflex afferents and transmission of inhibition to the VSCT.

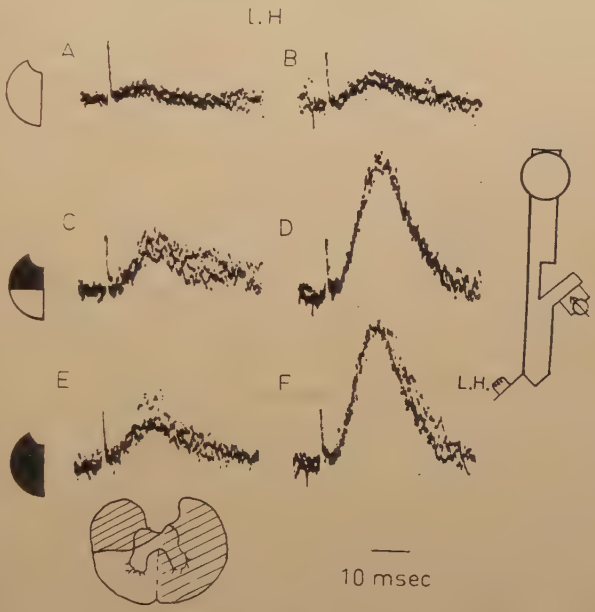

Fig. 5. – *Control of transmission to a ventrally located pathway by a descending system in the dorsal part of the other spinal half.*

Experimental conditions as shown in the schematic drawing with recording from the right spinal half on stimulation of the left hamstring nerve, in *A*, *C*, *E* at about 10 and in *B*, *D*, *F* at about 50 times threshold for the nerve. Decerebrate unanaesthetized cat. *A* and *B* were recorded with the left spinal half intact, *C* and *D* after section of the dorsal half of the left lateral funicle (histological control indicated in the drawing), *E* and *F*, finally after complete division of the cord.

The control of the ventral pathway is illustrated in Fig. 5 and 6. In Fig. 5 recording was made from the right spinal half on stimulation of the left hamstring nerve. Section of the dorsal half of the remaining left spinal half gives a large release (*C* and *D*) which does not further increase with complete transection of the cord (*E* and *F*). It should in particularly be noted that there is no increase of the submaximal mass discharge (*C* and *E*) after section of the ventral quadrant. In Fig. 6 it is demonstrated that transmission to the ventral pathway is controlled also from the same spinal half in which this pathway ascends. Records *A-D* were taken from the dissected left ventral quadrant after hemisection of the right spinal half. Hence the only connection between the upper and lower cord was the dorsal half of the left lateral funicle. After section of this remaining part a large release of late mass discharge occurred (*E-H*).

In Fig. 6 it should be noted that before complete section of the spinal cord, volleys in the nerves from the right side (*A* and *C*) evoked considerable late mass discharges, whereas volleys in the corresponding left nerves had very little action. In the spinal state (*E-H*) only small asymmetries were found. This finding was further analysed in experiments with separate recording of mass discharges from both ventral quadrants. With the dorsal half of the lateral funicles intact on both sides supramaximal hamstring volleys from either side did hardly evoke any late mass discharges in the right or left ventral quadrant. After section of the left dorsal quadrant, there was some release of mass discharge from the left nerve but not from the right. A further release to the spinal state occurred after section of also the right dorso-lateral quadrant and symmetrical discharges were recorded on both sides. It can therefore be concluded that although from either side the control is bilateral it is more effective with respect to transmission of effects from ipsilateral nerves. It is a task for future investigations to find where the cellbodies of the tract are located; probably they are located contralaterally to the half in which the axons ascend. Hypothetically it may be suggested that the inhibitory control is exerted on the interneurones mediating excitation to these pathways, and that these interneuronal pools are located mainly ipsilaterally to the entry of the nerves in the cord.

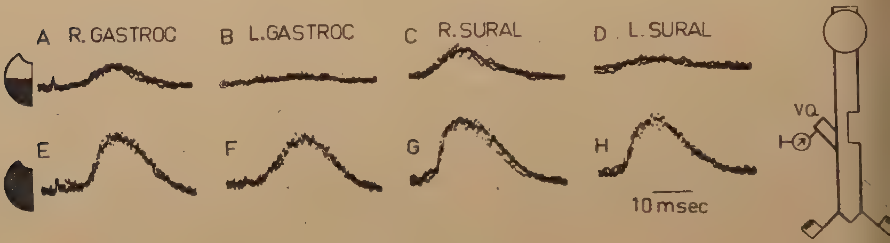

Fig. 6. – *Control of the transmission to a ventrally located ascending pathway by a descending system located in the dorsal part of same spinal half as the ascending pathway.*

Recording from the left dissected ventral quadrant on (supramaximal) stimulation of the right (*A*, *E*) and left (*B*, *F*) gastrocnemius-soleus nerves and right (*C*, *G*) and left (*D*, *H*) sural nerves. The cat was decerebrate and unanaesthetized. The right spinal half and the left ventral quadrant of the cord was sectioned. Upper records (*A*, *D*) were obtained before, lower corresponding records (*E*, *H*) after section of the remaining dorsal half of the left lateral funicle.

On the basis of this hypothesis the asymmetry in control after hemisection would indicate that the descending pathway on either side controls ipsilateral interneurones somewhat more effectively than contralateral ones.

With the interneurones of the flexion reflex it was not possible to demonstrate any difference in tonic inhibition exerted by ipsilateral and contralateral descending pathways (12), but it is probable that a slight asymmetry would have escaped notice since in this case the tonic inhibitory effect from the two sides was not compared in the same animal.

Transmission to the dorsal pathway activated by the ipsilateral flexion reflex afferents is also tonically inhibited by descending pathways in the dorsal half of the lateral funicles. Records *A-D* of Fig. 7 were obtained from the right dissected Flechsig's fasciculus, *A-C* with stimulation of the right hamstring nerve. In *D* the left hamstring nerve is stimulated and does not evoke any discharge. In *A* with intact left spinal half there is very little late mass discharge. After section of the dorsal half of the lateral funicle in *B* a release occurs which does not increase after section of the remaining ventral quadrant in *C*. In order to investigate if in addition there

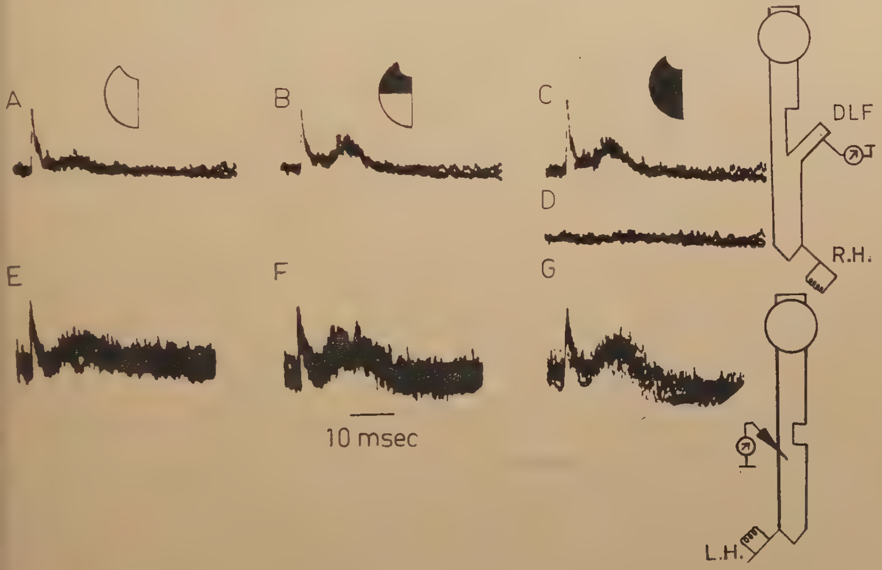

Fig. 7. – *Release of transmission to pathways in Flechsig's fascicle after localized spinal sections.*

In *A-D* the recording was made from the right dissected Flechsig's fascicle on stimulation of the right (*A-C*) and the left hamstring nerve (*D*). Records *E-G* were obtained in another experiment from a needle electrode superficially insterted in the left Flechsig's fascicle on stimulation of the left hamstring nerve. *A* and *E* show the discharges with intact left spinal half, *B* and *F* after section of the dorsal half of the left lateral funicle and *C*, *D* and *G* after complete division of the cord. Observe in *D* absence of discharge in Flechsig's fascicle on the right side on stimulation of left hamstring nerve.

is an ipsilateral control it was obviously not possible to record from the dissected Flechsig's fasciculus. Experiments were therefore made with the conventional technique of recording with needle electrodes (tip dimater of 10-20 μ). *E-G* of Fig. 7 were recorded in this way with the tip of the electrode inserted only superficially in the dorsal part of the lateral funiculus. It should be noted that the recorded potential is positive; a very localized recording is made in this way and there was never any effects recorded on stimulation of contralateral nerves. The potentials recorded with this technique resemble closely those recorded from the dissected Flechsig's fascicle. In the experiment of *E-G* the right spinal half was sectioned, *E* shows the discharge with intact left spinal half, *F* the release after section of the dorsal quadrant and *G* after complete spinal transection. The late mass discharge does not increase from *F* to *G*; hence there is an ipsilateral descending inhibitory control, like the contralateral one located in the dorsal part of the lateral funicle.

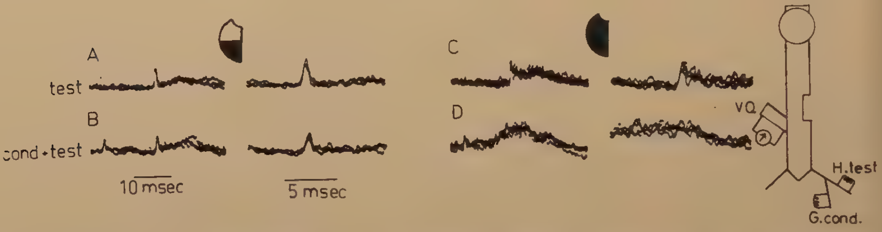

Fig. 8. – *Localization of descending paths suppressing the inhibitory actions exerted from high threshold muscle afferents on VSCT neurones.*

The testing VSCT discharge was recorded from the left dissected ventral quadrant on stimulation of the right hamstring nerve (*A* and *C*). In records *B* and *D* are shown the effects of a conditioning volley to the right gastrocnemius-soleus nerve (5 times threshold). Traces to the right in each record demonstrate the test VSCT discharge recorded simultaneously at faster speed. Records *A* and *B* were obtained in a decerebrated cat with only the left dorsal quadrant intact and *C* and *D* after section of this remaining connection.

The control of transmission of inhibition to VSCT was investigated in similar experiments. When in experiments of the type illustrated in Fig. 5 the dorsal part of the remaining intact spinal half was sectioned a release of inhibitory action on to VSCT occurred which did not increase when also the ventral quadrant was sectioned. The existence of a descending control on the side of VSCT is demonstrated in Fig. 8. After right hemisection of the cord the

VSCT discharge was recorded in the left dissected ventral quadrant, the dorsal quadrant remaining intact. The monosynaptic test discharge alone is shown in *A* and *C*. A conditioning volley from the right gastrocnemius-soleus nerve gave a slight inhibition when the left dorsal quadrant was intact but after section of it, the same conditioning volley gives complete inhibition in *D*.

As with the ventral pathway activated by the flexion reflex afferents asymmetries were found in the control of inhibitory actions to VSCT. Recording was made from both ventral quadrants and the dorsal half of the lateral funicle was intact on one side. Inhibitory action were evoked from contralateral nerves. Under these conditions inhibition was more effectively controlled to the VSCT of the hemisectioned side. It is possible that also in this case the interneurones are located mainly ipsilaterally to the entry of the nerves in the cord, and that the descending pathway on either side controls ipsilateral interneurones somewhat more effectively than contralateral ones.

4. *Methods for experimental handling of the descending control.* — It is of methodological interest that it is possible to block the supraspinal inhibitory control reversibly. In the experiment of Fig. 9, referred to above, the ventral quadrants were dissected for recording, the dorsal halves of the lateral funicles were intact and a thermode placed under them. The release of ascending mass discharge during cold block of the descending pathways is shown in *D* and *E* and the reappearance of the control after rewarming in *G* and *H*. In this experiment the cold block was applied more than 10 times without any loss of reversibility. Records *C*, *F* and *I* shows the baseline without stimulation, with the indication of a more intense resting activity in the spinal state. Subsequent comparison with the release occurring after spinal transection revealed that the release in *D* and *E* was complete.

Records *J* and *K* in Fig. 9 are from another experiment on a spinalcat. *J* shows the mass discharge in the dissected right ventral quadrant on stimulation of the left hamstring nerve. During repetetive stimulation of the dissected left dorsal half of the lateral funicle the late mass discharge due to activity in the ventral pathway activated by the flexion reflex afferents disappears entirely whereas the initial discharge in the VSCT is unaffected.

5. *Control from higher centres.* — The supraspinal control of transmission to the ventral pathways has been compared in animals with intact brain and after decerebration. Such an experiment is

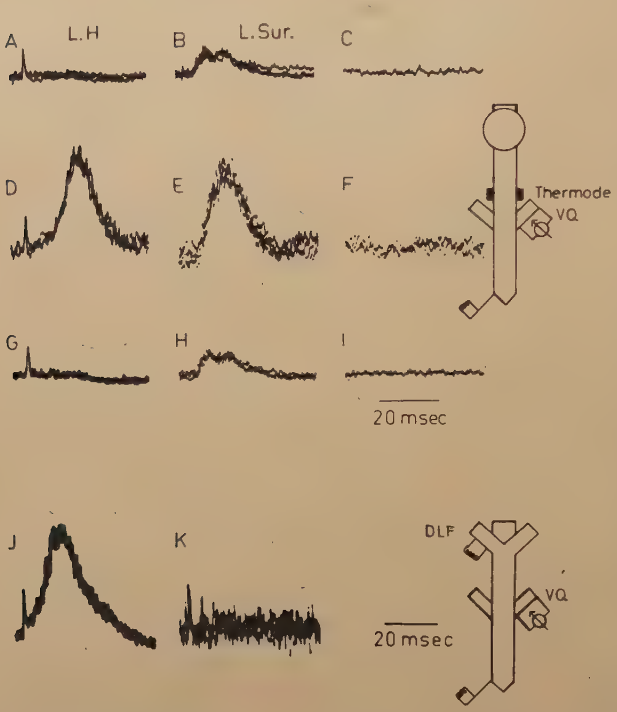

Fig. 9. – *Different experimental possibilities to interfere with the control of transmission to ventrally located pathways activated by the flexion reflex afferents.*

In the experiment of *A-I* the ventral quadrants were sectioned and dissected for a distance of about 20 mm and a thermode placed under the remaining dorsal part of the cord (dorsal column removed). Recording was made from the right ventral quadrant on stimulation of the left hamstring nerve (*A*, *D* and *G*) and the left sural nerve (*B*, *E* and *H*). Records *C*, *F* and *I* show basic activity without stimulation. *A-C* were obtained before, *G-I* after and *D-F* during cold block of the dorsal cord (0° C).

Records *J* and K were obtained in another experiment on a spinal cat. The ventral quadrants were sectioned and dissected for recording. At a more cranial level the dorsal halves of the lateral funicles were dissected for stimulation. In *J* and *K*, recorded from the right ventral quadrant, the left hamstring nerve was stimulated supramaximally; in *K* during repetetive stimulation of the dorsal half of the left lateral funicle. Observe that transmission of the late mass discharge is suppressed whereas the early VSCT discharge is unaffected.

illustrated in Fig. 10. The monosynaptic VSCT discharge (recorded in the right spinal half on stimulation of the left hamstring nerve) was used as test (shown alone in *D*, *H* and *L*). A conditioning stimulus was given to the left gastrocnemius-soleus nerve. The conditioning response alone is shown in *A*, *E* and *I* and conditioning plus testing in *B*, *F*, *J* and *C*, *G*, *K*, the latter records were obtained simultaneously as the former but with faster sweep speed. The conditioning volley alone gave a small late mass discharge in *A* when the brain was intact, after decerebration there was hardly any mass discharge, which shows that the inhibitory control of transmission was more intense. After spinal section the usual release occurred with a late mass discharge that was considerably bigger than when the brain was intact. Similar observations were made with transmission of inhibition to VSCT. In the intact state conditioning decreased the VSCT discharge by 35% (*B*, *C*, Fig. 10) after decerebration by 10% (*F*, *G*) and after section of the cord there was complete inhibition. Hence both with the control of transmission to the ventral pathway activated by the flexion reflex afferents and with transmission of inhibition to the VSCT there is a parallelism with the supraspinal inhibitory control of the flexion reflex interneurones, which are more effectively controlled in the decerebrate

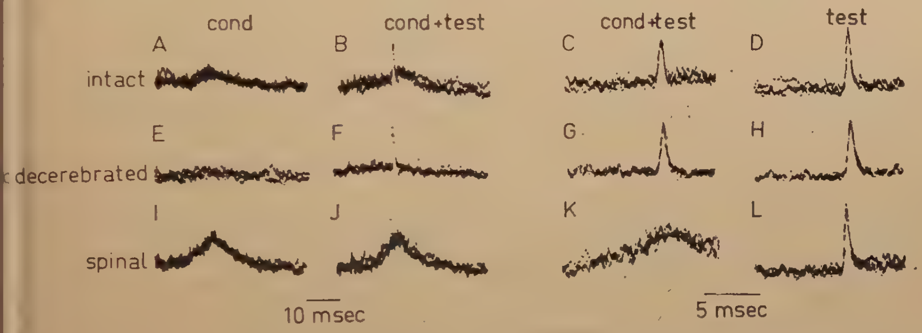

Fig. 10. – *Influence from higher centers on the inhibitory effects of high threshold muscle afferents on VSCT neurones.*

The monosynaptic testing VSCT discharge was recorded from the right spinal half on stimulation of the left hamstring nerve (unconditioned test discharge in *D*, *H* and *L*). The effect of a conditioning volley in the left gastrocnemius-soleus nerve on the test VSCT spike is shown in *B*, *F*, *J* and is recorded with faster sweep speed in *C*, *G*, *K*. Conditioning stimulus strength was 9.5 times threshold for the nerve. The discharges evoked by the conditioned volley alone is shown in *A*, *E* and *I*. Records *A-D* were obtained when the brain was intact, *E-H* after decerebration and *I-L* after section of the remaing left spinal half. Lightly anaesthetized preparation.

state than when the brain is intact. Further similarities are provided by the independence of this inhibitory control from cerebellum. This is illustrated in Fig. 11. The upper traces of the records in Fig. 11 were recorded from the right dissected spinal half on stimulation of the left gastrocnemius-soleus nerve (*A-C*) and of the left sural nerve (*D-F*). A and *D* show the discharges before and *B* and *E* during repetetive stimulation of the anterior cerebellum causing complete collapse of decerebrate rigidity. The release after spinal transection is shown in *C* and *F*. The records of Fig. 11 were chosen for illustration because in this experiment the supraspinal control of transmission was not very intense, hence a background would be provided to show either an increased or decreased effectiveness of the supraspinal inhibition. Neither was there any change in the supraspinal control in other experiments with more effective supraspinal inhibition of transmission than in Fig. 11. The lower traces in the records of Fig. 11 show that discharge in the nerve of post.biceps-semitendinosus and illustrate that the control of the flexor is also unaffected by cerebellar stimulation (12). Transmission to the ventral pathway was tested in several experiments before and after total ablation of the cerebellum but there was never any effect.

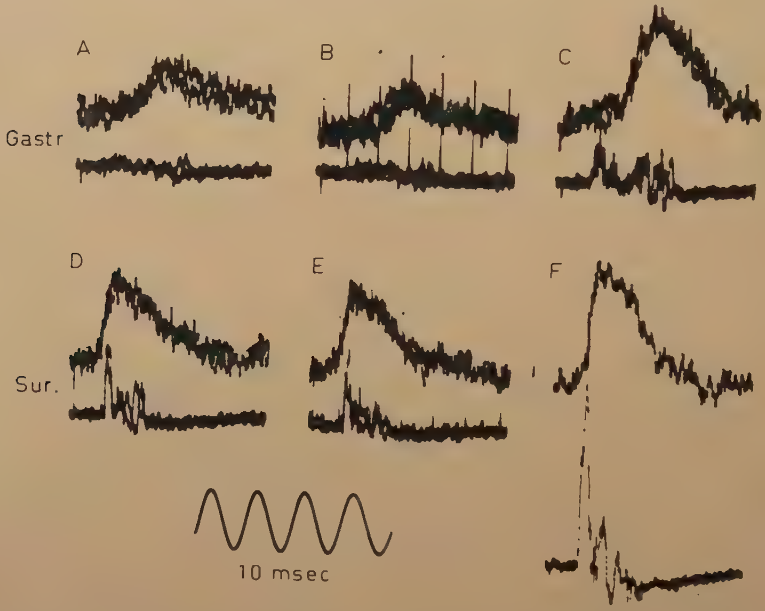

Fig. 11. – *Absence of effect from the anterior cerebellum on transmission to ventrally located pathways.*

The discharge in the upper traces were recorded from the right dissected spinal half on supramaximal stimulation of the left gastrocnemius-soleus nerve (*A*-*C*). The lower traces were recorded simultaneously from the left post. biceps-semitendinosus nerve. Decerebrate unanaesthetized preparation with the anterior cerebellum exposed for stimulation. The discharges in *B* and E were evoked during repetetive cerebellar stimulation. *C* and *F* show the release of late mass discharge and flexor reflex after section of the remaining left spinal half.

Similar experiments were made with respect to the control of transmission of inhibition to the VSCT. Likewise no change was found in the effectiveness of the supraspinal control either on stimulation or ablation of cerebellum. Bilateral ablation of the vestibular nuclei was made in two experiments and did not affect transmission to the ventral pathway. A full release to the spinal state was found after a lesion at obex; presumably the responsible centres are located in the reticular formation.

It is possible and perhaps even likely that a detailed analysis will reveal that the supraspinal control of transmission to these pathways in some way may be affected from cerebellum. The present experiments have served mainly to indicate that the supraspinal control of transmission to the pathways functions independently of centres responsible for decerebrate rigidity, although in both cases a release occurs with decerebration.

DISCUSSION

The present paper is introductory to a series of investigations aiming at disclosing the functional significance of a number of ascending spinal pathways, which in the spinal preparation have the feature in common, that they are strongly influenced by primary afferents which give rise to the flexion reflex. Three such pathways have been studied: 1) a dorsal pathway activated by the ipsilateral flexion reflex afferents (18); 2) a ventral pathway activated by these afferents from a bilateral receptive field (22); 3) the ventral spinocerebellar tract, receiving monosynaptic excitatory action from I *b* afferents and strong inhibitory action from the flexion reflex afferents (21).

With all these pathways the problem arises what type of messages they can convey. Since these pathways are influenced from skin, muscle and joint afferents they can neither be classified as proprioceptive nor as exteroceptive pathways, and their very wide receptive fields makes it difficult to understand how they can convey but very crude spatial information. On the other hand, it would be possible that they in some way are informative with respect to the flexion reflex. It has been demonstrated that the interneurones mediating the spinal flexion reflex actions from group II and III muscle afferents, from skin and from high threshold joint afferents are tonically inhibited from supraspinal centres (4, 12). In order to evaluate the relationship between the flexion reflex and the function of these asceding pathways it is important to know if in the decerebrate state transmission to them is subject to a similar tonic inhibition from supraspinal centres. It has now been found that this is the case. With both the ventral and the dorsal pathways receiving excitation by the flexion reflex afferents, transmission is often inhibited to a degree that hardly any discharge is evoked by supramaximal single volleys from muscle nerves; in neurones of the ventral spino-cerebellar tract only slight inhibitory effects are evoked by strong conditioning volleys from skin or muscle nerves compared with the complete inhibition found in the spinal state.

The finding that transmission to these sensory pathways is subject to an inhibitory influence from higher centres is not very surprising. A control of this type was first demonstrated by Hagbarth and Kerr (8) and has later been found and investigated in detail with a number of sensory pathways (7, 9, 10, 11, 23). In the present investigation it has also been shown that inhibitory actions exerted on cells of ascending tracts may be controlled from higher centres. Functionally this control of inhibition may be equivalent to the control of transmission of excitatory action because some pathways may forward information through inhibitory actions working against a background of resting discharge.

With the VSCT the supraspinal control of transmission of inhibitory action must be exerted on the interneurones mediating this inhibition. A similar situation may prevail with the control of the two pathways mediating excitation from the flexion reflex afferents. These excitatory actions are mediated via interneurones (18, 22) and it is highly probable that the tonic inhibitory control described in this paper is exerted on the interneurones and not on the cells of the axons constituting the ascending pathways. It will be demonstrated in forthcoming papers that these pathways are also governed by other systems of supraspinal control. For example, the ventral one of the pathways activated by the flexion reflex afferents receives monosynaptic excitatory action from the brain stem (Holmqvist, Lundberg and Oscarsson, unpublished), and the cells of the ventral spino-cerebellar tract can be inhibited from supraspinal centres (Oscarsson, unpublished). In order to advance in the field of supraspinal control it is necessary not only to work on defined ascending pathways but also to specify the controlling system.

The particular interest of the supraspinal control of transmission to pathways disclosed in this investigation, lies in the similarity with the control exerted on the flexion reflex interneurones, in connection with the fact that these pathways are influenced by the same afferents that give rise to the flexion reflex actions.

In both cases the descending pathways are located in the dorsal part of the lateral funicles. The tonic inhibition in more intense in the decerebrated preparation than in the animal with intact brain. Likewise there was no effect from cerebellar stimulation or ablation and the tonic inhibitory control remained after bilateral ablation of the vestibular nuclei. These similarities support the notion that there may be a relationship between these ascending pathways and the flexion reflex. For example, if transmission to the pathways and through the flexion reflex interneurones were inhibited by the same controlling system or by controls coupled in parallel, these pathways could forward information regarding the transmittability of the flexion reflex interneurones. It is of cause possible that the resemblances found may be superficial. The inhibitory control may serve more dynamically to select and restrict the receptive field to parts physiologically needed. Further experiments must be devised in order to test to which extent the inhibitory control is linked to control of reflex arcs.

Forthcoming reports will deal with the discharge in single axons of these pathways; in particular with adequate activation at different degrees of supraspinal control. Knowledge on the location of descending pathways and their organization has been a prerequisite for experiments of this type.

It is obvious that for the assessment of the functional significance of these pathways their termination must be known. Such experiments are in progress (19). Our general procedure has been first to define the pathways by their pattern of activation from the periphery and then to study how transmission is controlled from supraspinal centres. The results reported here have justified this approach. It would clearly have been very difficult to follow the further anatomical course of the three pathways in the traditional way by using the discharge evoked from the periphery as index; at least in the decerebrate preparation in which the supraspinal inhibitory control almost entirely prevents transmission to some of these pathways.

SUMMARY

A study has been made of the supraspinal control of transmission to three ascending spinal pathways influenced by the afferents that segmentally give rise to the flexion reflex actions.

Comparison of transmission in decerebrate and spinal cats revealed that in the decerebrate state transmission to three pathways is tonically strongly inhibited from supraspinal centres. The ventral spino-cerebellar tract receives inhibition from the flexion reflex afferents and it is concluded that the supraspinal tonic inhibition is exerted on interneurones mediating inhibition from the periphery. Two other pathways are excited by the flexion reflex afferents, and it is suggested that also in this case the supraspinal inhibitory control is exerted on interneurones mediating the excitatory effects from the periphery.

In the intact animal the supraspinal control of transmission to these pathways is less intense than in the decerebrate.

The tonic inhibition of the flexion reflex interneurones is neither affected when decerebrate rigidity collapses on stimulation of cerebellum nor after ablation of cerebellum and the vestibular nuclei.

With all three systems the descending pathways responsible for the tonic inhibition are located in the dorsal half of the lateral funicle and from each side a bilateral effect is exerted.

Reversible removal in the decerebrate animal of the supraspinal control of transmission to the pathways has been achieved by blocking conduction (cold) in the dorsal half of the lateral funicles. In spinal animals activation of the descending control to ensure complete suppression of transmission results on stimulation of either dorsal half of the lateral funicle.

The function of the pathways is discussed on the basis of the findings that they are influenced by the same afferents wich give rise to the flexion reflex and that the organization of the supraspinal inhibitory control of transmission to the pathways and of the flexion reflex interneurones are very similar.

ACKNOWLEDGEMENT

This work was supported by a grant from the Swedish Medical Research Council. Technical assistance was given by Mr. E. Eide and Miss K. Ebbesson.

REFERENCES

1. Bishop, P. O. and Collin, R. Steel microelectrodes. *J. Physiol.*, *112*: 8-10 P, 1951.
2. Eccles, R. M. and Lundberg, A. Significance of supraspinal control of reflex actions by impulses in muscle afferents. *Experentia*, *14*: 197, 1958.
3. Eccles, R. M. and Lundberg, A. Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. *Arch. ital. Biol.*, *97*: 199-221, 1959.
4. Eccles, R. M. and Lundberg, A. Supraspinal control of interneurones mediating spinal reflexes. *J. Physiol.*, *147*: 565-584, 1959.
5. Green, J. D. A simple micrelectrode for recording from the central nervous system. *Nature*, *182*: 962, 1958.
6. Grundfest, H., Sengstaken, R. W., Oettinger, W. H. and Gurry, R. W. Stainless steel microneedle electrodes made by electrolytic pointing. *Rev. sci. Instrum.*, *21*: 360-361, 1950.
7. Hagbarth, K.-E. Centrifugal mechanisms of sensory control. *Ergebn. Biol.*, in the press.
8. Hagbarth, K.-E. and Kerr, D. I. B. Central influences of spinal afferent conduction. *J. Neurophysiol.*, *17*: 295-307, 1954.
9. Hernàndez-Peón, R. Central mechanisms controlling conduction along central sensory pathways. *Acta neurol. latinoamer.*, *1*: 256-264, 1955.
10. Hernàndez-Peón, R. and Scherrer, H. Inhibitory influence of brain stem reticular formation upon synaptic transmission in trigeminal nucleus. *Fed. Proc.*, *14*: 71, 1955.
11. Hernàndez-Peón, R., Scherrer, H. and Velasco, M. Central influences on afferent conduction in the somatic and visual pathways. *Acta neurol. latinoamer.*, *2*: 8-22, 1956.
12. Holmqvist, B. and Lundberg, A. On the organization of the supraspinal inhibitory control of interneurones of various spinal reflex arcs. *Arch. ital. Biol.*, *97*: 340-356, 1959.
13. Holmqvist, B., Lundberg, A. and Oscarsson, O. Functional organization of the dorsal spino-cerebellar tract in the cat. V. Further experiments on convergence of excitatory and inhibitory actions. *Acta physiol. scand.*, *38*: 76-90, 1956.
14. Holmqvist, B., Lundberg, A. and Oscarsson, O. The relationship between the flexion reflex and certain ascending spinal pathways. *Experientia*, *15*: 195, 1959.
15. Job, C. Ueber autogene Inhibition und Reflexumkehr bei spinalisierten und decerebrierten Katzen. *Plügers Arch.*, *256*: 406-418, 1953.
16. Jouvet, M. and Desmedt, J.-E. Contrôle central des messages acoustique afférents. *R. C. Acad. Sci.*, *243*: 1916-1917, 1956.
17. Laporte, Y., Lundberg, A. and Oscarsson, O. Functional organization of the dorsal spino-cerebellar tract in the cat. I. Recording of mass discharge in dissected Flechsig's fasciculus. *Acta physiol. scand.*, *36*: 175-187, 1956.
18. Laporte, Y., Lundberg, A. and Oscarsson, O. Functional organization of the dorsal spino-cerebellar tract in the cat. II. Single fibre recording in Flechsig's fasciculus on electrical stimulation of various peripheral nerves. *Acta physiol. scand.*, *36*: 188-203, 1956.
19. Lundberg, A. and Oscarsson, O. Identification of a third subdivision of the dorsal spino-cerebellar tract. *Experientia*, *15*: 195, 1959.
20. Oscarsson, O. Functional organization of the ventral spino-cerebellar tract in the cat. I. Electrophysiological identification of the tract. *Acta physiol. scand.*, *38*: 145-165, 1956.

21. Oscarsson, O. Functional organization of the ventral spino-cerebellar tract in the cat. II. Connections with muscle, joint and skin nerve afferents and effects on adequate stimulation of various receptors. *Acta physiol. scand.*, *42*, suppl. *146*: 1-107, 1957.
22. Oscarsson, O. Further observations on ascending spinal tracts activated from muscle, joint and skin nerves. *Arch. ital. Biol.*, *96*: 199-215, 1958.
23. Scherrer, H. and Hernàndez-Peón, R. Hemmung postsynaptischer Potentiale im Nucleus gracilis. *Pflügers Arch.*, *267*: 434-445, 1958.

SOME OBSERVATIONS ON THE RETINAL POTENTIALS OF THE FISH

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INTRODUCTION

Svaetichin recorded from an isolated retina with a capillary microelectrode a negative potential shift, which he considered to be the resting membrane potential of single cones, and a further negative (hyperpolarizing) deflection following illumination, which he originally called "cone action potential" (11, 13).

Tomita (14), however, by measuring the depth in the retina from which the maximal response was obtained concluded that this potential originates in a region proximal to the receptor layer. Tomita (14) also questioned the assertion that this potential was recorded intracellularly on the basis of the observation that the response could be recorded with large (5 μ) pipettes.

Histological localization of the electrode tip (7, 8, 10, 15) confirmed the inference that Svaetichin's hyperpolarizing potential originates in the secondary neuronal layer, but no conclusion could be reached with this techinique concerning its intra- or extracellular origin.

In 1958, Dr. C. Spyropoulos (unpublished) found that there is no correlation between DC level and the amplitude of the response to light. He also noted that displacement of the electrode (up and down and sidewise) of more than 100 μ was frequently possible without abolishing the response. In agreement with MacNichol (6), Spyropoulos also found that anodal and cathodal polarization applied through the microelectrode did not alter the recorded potential.

The experiments described in the present paper were performed with the purpose of re-examining whether hyperpolarizing responses to light can be recorded only after a negative steady potential (membrane potential?) has been obtained, and whether the responses to light can be influenced by electric currents.

METHODS

The inverted retina of the common goldfish was used. The receptor side of the retina was exposed by removing the sclera and choroid in the same manner described elsewhere (9).

The preparation was mounted with the receptor side up on a reference electrode which consisted of Ag-AgCl and cotton soaked in Ringer. A capillary microelectrode of less than *1 μ* tip diameter was used. The resistance of the electrode was monitered throughout the experiment by applying a square pulse to the electrode through a small capacitor after each light stimulus (Fig. 1). By this method the amplitude of applied pulse was displayed on the cathode ray oscilloscope and measured the resistance of the electrode (Figs. 2 and 4). The cathode follower designed by Bak (1) and a Tektronix 112 DC amplifier were employed.

The potential drops evoked by currents through a microelectrode were balanced by means of a bridge circuit (Fig. 1, and also see 4). The light source was a Sylvania C-100 Concentrated Arc Lamp. The whole eye was illuminated and a mechanical shutter was used for obtaining light flashes of the desired duration.

RESULTS

1. *Surface positive response.* — When the electrode made contact with the receptor side of the retina, a small surface positive response was often observed following illumination. This response had a larger amplitude (up to 2 mV) when the pigment epithelium was intact. As the electrode was further advanced into the retina, the positive response became gradually smaller in amplitude. After this, in some cases, a small negative response, unaccompanied by DC shift, appeared. Small advancement of the electrode from the depth where the first (positive) response diminished, or the second (small negative) response was obtained, often caused a fairly large negative DC shift and the large sustained negative response to light described by Svaetichin and by other investigators. In accordance with recent terminology (12) this response will be called here the graded photic response (g.p.r.).

The small positive response recorded from the surface was sometimes considered to be the extracellular recording of the large negative potential, g.p.r. (7, 9). However, this is doubtful because

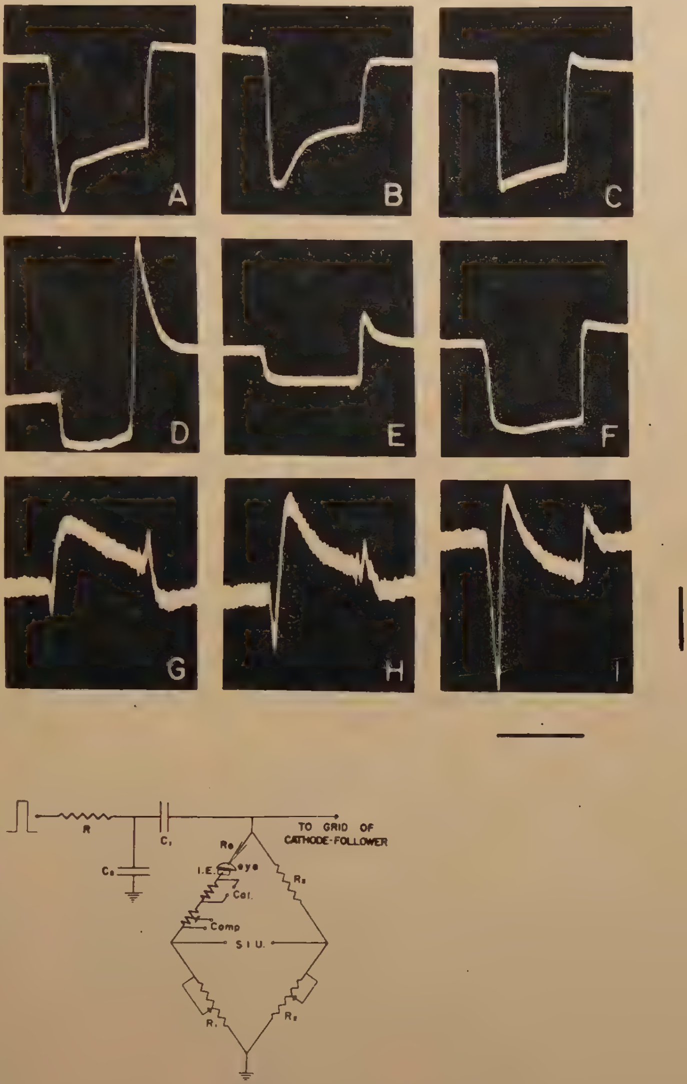

Fig. 1. – *Various responses recorded with microelectrodes.*

The magnitudes of the associated negative DC shifts are 15 (*A*), 10 (*B*), 8 (*C*), 23 (*D*), 32 (*D*), 32 (*E*), 35 (*F*), 30 (*G*), 35 (*H*), and 38 (*I*) mV. Calibration: 10 mV for *A-F*, 3,5 mV for *G-I*. Time line: 1 sec. Upward deflection indicates positivity of the microelectrode in this and in the following figures. Diagram at the bottom shows the arrangement used for measuring the electrode resistance and for passing currents. R: 160 KΩ; C_1: 1.2 μ μ F; C_2: 0.5 μ F; R_1: 10 KΩ; R_2: 100 KΩ; R_3: 500 MΩ; Cal: 100 Ω (calibrator); Comp.: 100 Ω (compensator). R_e: the resistance of the electrode was between 10 and 50 MΩ while recording. S.I.U.: radio frequency stimulus isolation unit which was used for passing currents. I.E.: an Ag-AgCl plate which served as an indifferent electrode.

the g.p.r. appeared after the small positive response had diminished, or had reversed to a small negative potential without negative DC shift.

2. *Various negative or complex responses.* — Different types of negative responses can be recorded following illumination with white light (Fig. 1). In some instances these responses changed their configuration as time elapsed. Some of these responses were susceptible to light and dark adaptation, and it was frequently observed that their fast negative component (see Fig. 1 *A*, *B*, *G*, *H*, *I*) augmented after several seconds interruption of light stimulation.

In this paper all responses recorded in the deeper layers (roughly more than 100 μ below the surface) were classified as g.p.r., if the sustained negative component was predominant (Fig. 1 *A-F*). The more complex responses illustrated in Fig. 1 *G-I* were not considered in this study.

3. *Relation between g.p.r. and DC shift.* — It is generally accepted that a large negative DC shift is regularly associated with the g.p.r. In order to test this view, potentials were recorded while the electrode was moved down slowly from the receptor side of the retina (Fig. 2). In these experiments a flash of light of one-second duration was delivered approximately once every 3.5 sec. Manipu-

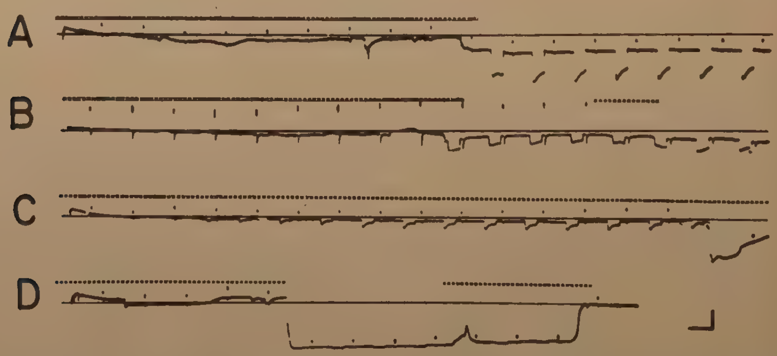

Fig. 2. – *The sequence of the potentials while the electrode was traversing the retinal layers.*

Upward pulses which appear following each light flash indicate the resistance of the electrode. Horizontal solid and dotted lines show the zero levels of DC potential, and the advancement of electrode, respectively. Vertical line at the right lower corner: 20 mV for voltage calibration, 40 MΩ for the electrode resistance. Time line: 2 sec.

lation of the electrode was interrupted when the maximal response was recorded. The sequence of the events in *A* is the same as that referred to previously as typical. *B* and *C* are examples showing that a sudden change of negative DC potential is not essential for the g.p.r.; this is particularly evident in *C* where following a negative DC shift the response disappeared. *D* is an example of a small response with a large DC shift.

In Fig. 3 the amplitude of the responses to light recorded in different experiments is plotted against the magnitude of the associated DC shift which was taken with respect to the potential level recorded at the surface of the retina. It is seen in this figure that g.p.r.'s of over 20 mV amplitude were obtained with DC shifts between 10 and 40 mV and g.p.r.'s of over 10 mV with shifts from 0 to 45 mV.

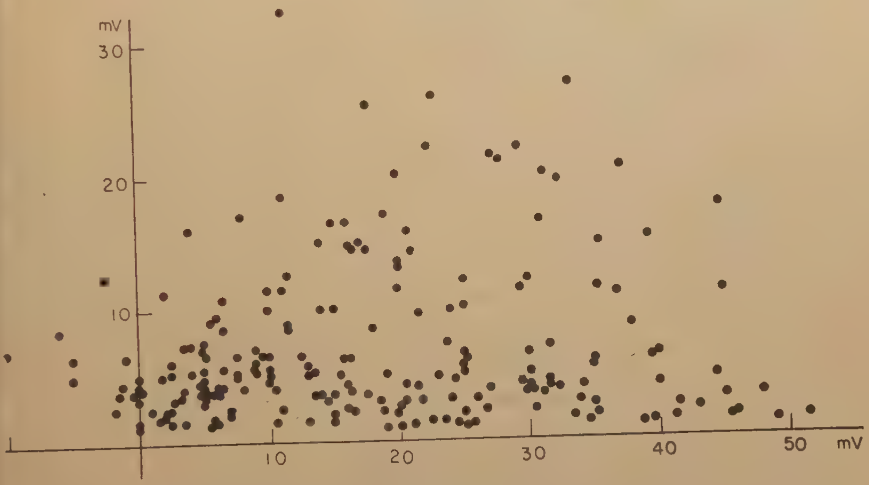

Fig. 3. – *Relationship between amplitude of the g.p.r. (ordinate) and the associated negative DC shift (abcissa).*

4. *Effect of electric current through the microelectrode.* — Slow potentials of junctional regions (endplate potentials and synaptic potentials) and of receptor organs (generator potentials) are supposed to originate as a consequence of a change of permeability of the membrane of the cell in which the potential occurs, and it has been suggested (7) that the g.p.r. of fish retina is produced by mechanisms similar to those giving rise to synaptic potentials. Potentials ori-

ginating as a consequence of permeability change to certain ions would be expected to be modified when the membrane potential is changed. Current through the microelectrode should modify the membrane potential and, thus, the g.p.r., if the microelectrode is intracellular.

For this experiment, large g.p.r.'s superimposed upon steady negative DC potentials of 10 to 30 mV were used. DC currents were passed in either direction using the Wheatstone's bridge to balance out the potential drop produced by currents. No significant difference in amplitude was observed with currents up to 10^{-8} A (Fig. 4). This finding is in agreement with results obtained by MacNichol (6), C. Spyropoulos and P. Gouras (personal communication).

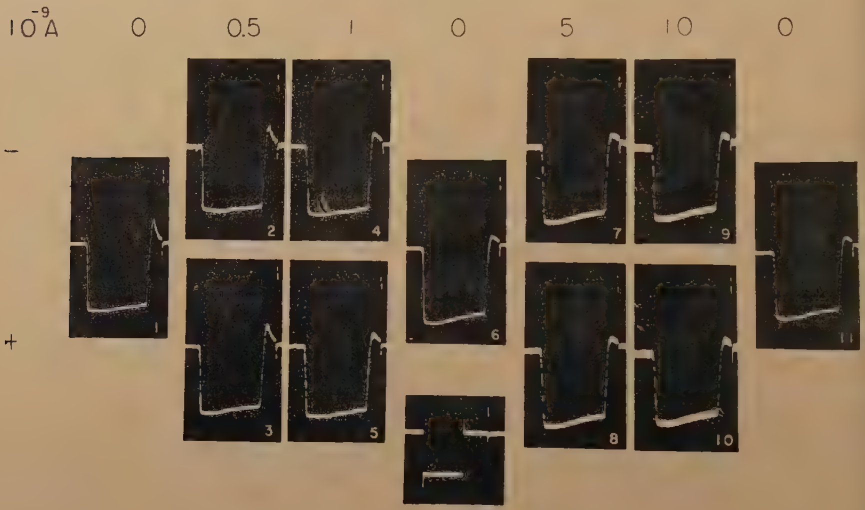

Fig. 4. – *The effect of currents upon the g.p.r.*

Records were taken from 1 to 11. Outward currents (electrode positive) were marked with +. Resistance of the electrode was measured immediately after the cessation of the light. Downward square wave shown in the middle lower photograph is 10 mV calibration, and the following upward pulse measures electrode resistance (20 MΩ).

In other experiments, square pulses of 10 msec duration, repeated 10 times every second, were used instead of steady currents, the bridge being balanced in darkness for the DC component of the pulse. As might be expeted from the previous result, no bridge unbalance took place following illumination when a g.p.r. was recorded. A slight bridge unbalance was sometimes superimposed upon the response, especially when the pigment epithelium remained intact (Fig. 5 *A*). However, the duration of umbalance was not only longer than that of the g.p.r. but it could also be recorded after withdrawal of the electrode from the region where the g.p.r. was obtained (Fig. 5 *B*). This result may be explained by a change in the resistance of the retina due to illumination (see 2).

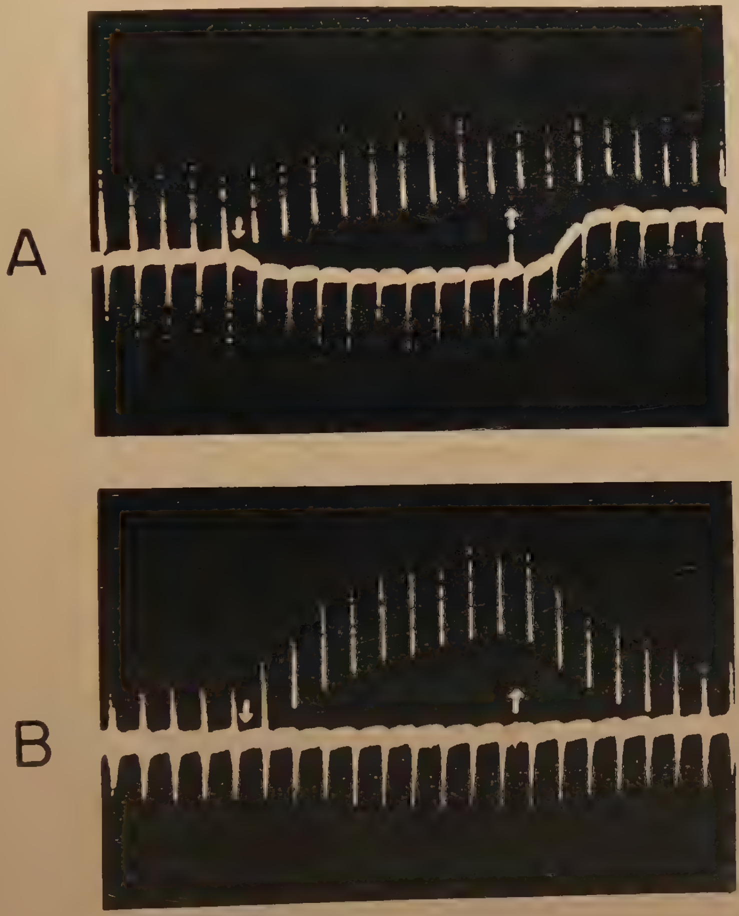

Fig. 5. – *Bridge unbalance due to illumination.*

Bridge unbalance is revealed by increased spacing between vertical transients. Arrows show on and off of the illumination.

A: Records obtained while g.p.r. was recorded. *B*: Same illumination. Record obtained after electrode had been withdrawn and was located on receptor surface of retina. (Surface positive response not visible due to low gain).

Although the resistance change described above appeared to be unrelated to the g.p.r., a few experiments were performed to investigate its origin.

A symmetrical recording system consisting of a pair of similar microelectrodes and of a push-pull cathode follower input stage was employed in order to cancel out the possible electromotive forces due to illumination. A current was passed through a pair of Ag-AgCl-agar Ringer gross electrodes in series with two 5 MΩ resistors. The potential drop evoked by the current across the tissue was measured with a pair of microelectrodes placed in between the two gross electrodes (Fig. 6).

Since no potential difference occurred between the two microelectrodes following illumination, and since the current was constant, any potential change occurring under these conditions must be ascribed to a resistance change.

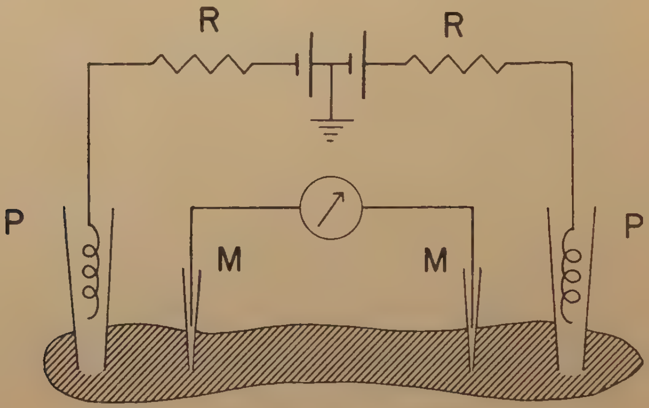

Fig. 6. – *Arrangement of electrodes for measuring resistance changes.*

M: Microelectrode for recording. *P*: Ag – AgCl – agar Ringer electrode for passing current. *R*: 5 MΩ. Shaded area indicates the preparation.

Upon illumination a change in the potential drop, proportional to current intensity, could be recorded from any tissue, the pigment epithelium showing the most marked changes. Pigment epithelium scraped off the eye behaved also in the same manner. The time course of this potential change was similar to that of the bridge unbalance mentioned above and its direction revealed a decrease of the resistance of the tissue.

This may be a purely physical phenomenon; the decrease in resistance may be largely due to the temperature rise of the tissue fluid following illumination.

DISCUSSION

It has been stated that a large negative DC shift is regularly associated with the g.p.r., and that the greater the negative DC shift the greater the g.p.r. (11,14). This observation has been considered to be important evidence for the view that the g.p.r.'s are recorded intracellularly. However, there is some disagreement on the value of the DC shift recorded prior to the g.p.r. (40-50 mV, Svaetichin, 11; 40 mV, Svaetichin, 12; 20-50 mV, Motokawa *et al.*, 9; 10-30 mV, MacNichol and Svaetichin, 7; 30 mV, Oikawa *et al.*, 10). In the present experiment, however, no relation between DC shift and g.p.r. could be found (Fig. 3). It should be noted that, whereas some scatter could be expected because of possible damage to the cell or change in the tip potential of the electrode, a reasonably good correlation between membrane and action potentials was found in other structures (e.g., Frank and Fuortes, 3).

In some cases, gradual shift of DC level (positive or negative) occurring spontaneously or following movement of the electrode did not affect the amplitude of the response to illumination or, conversley, gradual increase or decrease in the amplitude of this response was not associated with a change in DC potential. Therefore it may be concluded that only a poor correlation exists between DC shift and g.p.r.

Moreover, the following observations were made in this study: 1) no extracellular recording could be obtained just before DC shift occurred; 2) current through the microelectrode did not appreciably alter the recorded g.p.r.; 3) microelectrode movement did not necessarily change the recorded g.p.r.; 4) the g.p.r. could be recorded with large ($3\,\mu$) electrodes.

The first observation could be explained assuming that the structure responsible for g.p.r. production is activated all at the same time, as one might expect to occur in a cell without precesses. In these conditions, no extracellular currents would be recorded during activity.

The other observations appeared to require that g.p.r.'s are recorded from a large "space" and that the "membrane" across which the potential develops has low total resistance. The technique employed in the present study could not have revealed a change of resistance of less than 0.1 MΩ. Thus, it is possible that g.p.r. originates as a consequence of a conductance change of less than this value.

Alternatively, the g.p.r. could have a different origin, for instance, that of the potentials recorded by Lundberg from salivary glands (5).

Knowledge of the structure of the fish retina is still insufficient to decide whether or not some of the cells have properties satisfying the above requirements. It appears there are in the fish retina large horizontal cells located in the region from which g.p.r.'s are recorded. These cells are generally considered the most probable site of origin of g.p.r.'s (7, 8, 10); the possibility, however, that the g.p.r. may be recorded from extracellular spaces cannot be excluded at present.

SUMMARY

1) Properties of the graded photic responses (g.p.r.) of the retina of the fish have been studied by means of a capillary microelectrode.

2) No relation between DC shift and the g.p.r. could be found.

3) No extracellular current corresponding to the g.p.r. could be recorded.

4) Electric currents through the microelectrode did not modify the recorded g.p.r.

5) The g.p.r. could be recorded with large (3 μ) electrodes.

6) It is concluded that g.p.r.'s are recorded from a large " space ", either a large cell or an enclosed extracellular space.

ACKNOWLEDGEMENT

The author wishes to thank Dr. M. G. F. Fuortes for his helpful advice and valuable discussion.

REFERENCES

1. BAK, A. F. A unity gain cathode follower. *EEG clin. Neurophysiol., 10*: 745-748, 1958.
2. BRINDLEY, G. S. The passive electrical properties of the frog's retina, choroid, and sclera for radial fields and currents. *J. Physiol., 134*: 339-352, 1956.
3. FRANK, K. and FUORTES, M. G. F. Potentials recorded from the spinal cord with microelectrodes. *J. Physiol., 130*: 625-654, 1955.
4. FRANK, K. and FUORTES, M. G. F. Stimulation of spinal motoneurones with intracellular electrodes. *J. Physiol., 134*: 451-470, 1956.
5. LUNDBERG, A. Secretory potentials and secretion in the sublingual gland of the cat. *Nature, 177*: 1080-1081, 1956.
6. MACNICHOL, E. F., Jr. Electrophysiology of the visual system. Discussion. *Amer. J. Ophthal., 46*: 58, 1958.
7. MACNICHOL, E. F., Jr. and SVAETICHIN, G. Electrical responses from the isolated retinas of fishes. *Amer. J. Ophthal., 46*: 26-46, 1958.
8. MITARAI G. The origin of the so-called cone potential. *Proc. Japan Acad., 34*: 299-304, 1958.
9. MOTOKAWA, K., OIKAWA, T. and TASAKI, K. Receptor potential of vertebrate retina. *J. Neurophysiol., 20*: 186-199, 1957.
10. OIKAWA, T., OGAWA, T. and MOTOKAWA, K. Origin of so-called cone action potential. *J. Neurophysiol., 22*: 102-111, 1959.

11. SVAETICHIN, G. The cone action potential. *Acta physiol. scand.*, *29*, suppl. *106*: 565-600, 1953.
12. SVAETICHIN, G. Electrophysiology of the visual system. Discussion. *Amer. J. Ophthal.*, *46*: 58-59, 1958.
13. SVAETICHIN, G. and JONASON, R. A technique for oscillographic recording of spectral response curves. *Acta physiol. scand.*, *39*, suppl. *134*: 3-16, 1956.
14. TOMITA, T. A study on the origin of intraretinal action potential of the cyprinid fish by means of pencil-type microelectrode. *Jap. J. Physiol.*, *7*: 80-85, 1957.
15. TOMITA, T., MURAKAMI, M., SATO, Y. and HASHIMOTO, Y. Further study on the origin of the so-called cone action potential (S-potential). Its histological determination. *Jap. J. Physiol.*, *9*: 63-68, 1959.

Arch. ital. Biol., 98: 81-91, 1960.

ALPHA TYPES OF "RELEASE" STUDIED IN TENSION-EXTENSION DIAGRAMS FROM CAT'S FORELIMB TRICEPS MUSCLE[1]

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INTRODUCTION

It is known that skeletal muscle in reflex action faithfully reflects the state of its motor centre upon which excitatory and inhibitory influences converge from various receptive fields and supraspinal centres. Sometimes facilitatory, sometimes inhibitory influences prevail, but generally the two forces are simultaneously in action and partially cancel out, in proportion to their relative strengths.

An analysis of the mechanisms which underlie postural tone, makes clear that the extensor tonus is reflexly supported, at least in part, by impulses arising in the nuclear bag receptors of the muscle spindles. However the activity of the extensor motoneurones is also influenced by tonic autogenetic inhibition from Golgi tendon organs and probably also from the myotube (flower-spray) endings of the muscle spindles of the same muscle (for a summary, see 13). It is known that the α-motoneurones of extensor muscles of the forelimb are controlled by the following tonic inhibitory paths: 1) ascending reflex inhibition whose existence is proved by the release of the extensor motoneurones after postbrachial transection of the spinal cord (the well-known Schiff-Sherrington phenomenon, 30); 2) cerebellar inhibition, whose presence is shown by the increase of the extensor tone after cerebellectomy or bilateral destruction of the fastigial nuclei (cf. 2); 3) crossed reflex inhibition, proprioceptive in origin (25, 26, 27), whose existence is demonstrated by the release of extensor tone following deafferentation of the contralateral limb (38, 3, 4, 5), as well as by reversion of the postural asymmetry produced by unilateral destruction of the fastigial nucleus when the hypertonic limb is deafferented (25).

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[1] This work has been supported by grants to the Nobel Institute from the Swedish Medical Research Council and the Rockefeller Foundation. The author worked on a stipend from the Office of Scientific Research of the Air Research and Development Command, United States Air Force European Office, under Contract AF 61 (052)-115.

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The present work on forelimb stretch reflexes in extensors is mainly devoted to measurements of " release " after postbrachial transection of the spinal cord, cerebellectomy and contralateral deafferentation. It is based on evaluation of tension-extension diagrams. From such diagrams it is possible to measure thresholds of stretch reflexes as well as myotatic loop gain, which is the amount of myotatic reflex tension (output) produced per unit extension (input) in grams per millimetre (14,21). Our analysis of the relation between tension and extension of the triceps muscle has also included deafferented forelimbs. In this case the force developed during stretch of the muscle in excess of passive tension is unmodified by muscular afferents and due wholly to a constant tonic output through α-motoneurones. Such experiments therefore show what a constant output produces by itself owing to the mechanical properties of activated muscular tissue. If the deafferentation is carried out at the end of an experiment, it is also possible to deduce from the tension-extension diagram how much excitation the muscle end organs had contributed to the total, as long as they were connected with the spinal cord.

METHODS

The experiments were performed on 18 cats decerebrated at precollicular or intercollicular level. Decerebration was performed under ether anaesthesia, without clamping the vertebral arteries and with occlusion of both common carotids. The surface of the cerebellum was protected from cooling and drying. The precollicular decerebrate cats showed bilateral myosis, which could be followed during the whole experiment and served as a control of the functional integrity of the midbrain; these animals had a low degree of extensor rigidity of the forelimbs. On the other hand the intercollicular decerebrate animals exhibited a high degree of rigidity of the forelimbs. None of the cats had opisthotonus, which should be considered a sign of cerebellar deficiency (2).

In order to expose the spinal cord at brachial level (C V, Th II) as well as at postbrachial level (Th XII-LI) laminectomy was made. Exposure of the spinal cord took place 2-3 hours before the experiment started. The left forelimb was used. The caput longum of M. triceps brachii (extensor of the elbow) was isolated because it is a muscle especially affected by the decerebrate rigidity (32). All other muscles in the limb were paralyzed by nerve section or tying. The median, ulnar, musculocutaneus and medial cutaneous nerves were cut at the level of the axilla, while the radial nerve was sectioned distal to the branches of the triceps group. Stimulating electrodes were applied to the radial nerve and the proximal stump of the median or ulnar nerves of the ipsilateral side.

The animal laid prone, its head being fixed in symmetrical position by the Horsley-Clarke apparatus. The main fixation of the forelimb was by two twist drills which were inserted into the humerus just below the head and just above the epicondyle's fixation to upper and lower end respectively of the humerus. The scapula was held by one brass bone-clamp applied to the metacromion. The drills and the bone-clamp were held by joint-clamps to a steel upright, fixed to the top of a heavy all-metal experimental table. The muscle was attached by light metal hooks to the strain-gauge myograph. Zero tension at resting length could be adjusted to within 10 g. In all our experiments the muscle was submitted to stretches of 14 mm. The muscle was pulled out by a strong spring damped in oil and provided with an adjustable leak for different speeds. In order to study the tonic and phasic properties of the stretch reflex slow and fast rates of stretch were used. Rate of pull was linear and could be photographed whenever wanted (see Fig. 5 *B*). Electromyographic control of average output through bipolar Ag-AgCl wire electrodes at a distance of 4 mm was always used. Occasionally single motor units were recorded.

Temperature control was based on thermocouples in the rectum, and under the skin of the arm extensors. Observations began not less than one hour after ether anaesthesia had been discontinued. The postbrachial transection of the spinal cord, preceded by local novocainization, was performed at Th XII. Deafferentation was carried out extradurally, in acute experiments and a local anaesthetic was applied to the central ends of the cut dorsal roots. Complete cerebellectomy was performed by suction, while unilateral or bilateral destruction of the fastigial nuclei was produced electrolytically through electrodes orientated with the Horsley-Clarke stereotaxic instrument. The extent of the fastigial lesions as well as the integrity of neighbouring deep nuclei and of brain stem structures were controlled on serial Weil and Nissl stained sections.

In order to avoid fatigue of the muscle the measurements of tension were taken at fixed intervals of 5 min. Before recording the stretch reflex, the precaution was taken to stimulate the radial nerve by a single shock. This makes the muscle taut. Since the present work is concerned with active postural tension, the passive tension due to extension as such must be deducted from the total after suppression of the stretch reflex by inhibition, as achieved by electrical stimulation of the ipsilateral median or ulnar nerves. These nerves are known to inhibit the stretch reflex of the extensor muscles of the forelimb (9, 30). However, against large extensions inhibitions may not be complete and occasionally nerve stimulation may produce mixed effects. Passive tension has therefore been regularly measured after denervation. In this case the tension curve of the paralytic muscle was taken within half an hour or so of total severance of its nerve supply. A detailed discussion of errors in measuraments of active reflex tension has been given by Granit (14).

RESULTS

1. *Passive tension and myotatic loop gain of caput longum tricipis in the decerebrate animal.* — Fig. 1 shows the average passive tension measured in 10 animals of average weight 3.5 kg. The tensions developed at 14 mm extension ranged from 490 to 1350 g with an average of 800 g. While the passive tension tends to be approximately proportional to log. extension the myotatic or active tension is usually proportional to extension, as has been found previously by Granit (14) and Matthews (21) working on cat's soleus. Sometimes two straight lines of different slope are obtained (see below). This finding (see 14, 21) can be explained according to Granit (14) on the basis of different factors such as zero error, onset of full effect of series elasticity at a certain tension or a late start of recruitment of fresh motoneurones. Beyond 8-10 mm there is sometimes a flattening of the curve which is likely to be due to autogenetic γ inhibition (16, 11, 14) as well as to autogenetic α inhibition brought about by the Golgi tendon organs (see 13). However, the flattening of the tension curve for high extensions may also in part be a purely mechanical consequence of overstretch (Fig. 13).

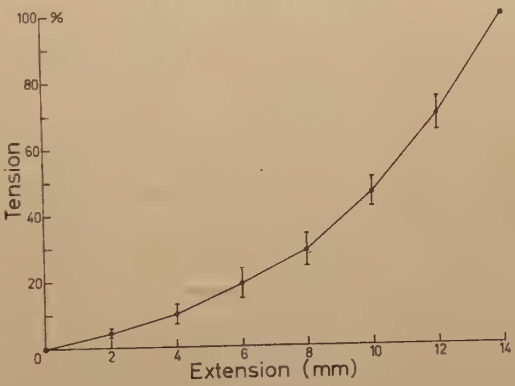

Fig. 1. – *Relative passive tension of caput longum tricipis plotted against mm of extension.*

Average values of measurements from 10 different experiments. The vertical lines are drawn on the basis of mean variations. The absolute values of passive tension at 14 mm of extension were 490, 555, 570, 650, 720, 740, 900, 950, 1080, 1350 g.

An analysis of the linear portion of our curves in the decerebrate condition indicates that the slope changes according to the level of decerebration. It is well known that the intercollicular decerebrate cat shows more forelimb rigidity than the precollicular animals. The average slope measured during tonic stretch reflexes (duration of stretch 18.5 sec) in precollicular cats was only 23 g/mm with a maximum reflex tension of 270 g, while in the intercollicular animals it was 107 g/mm, with a maximum tension of 850 g. Corresponding differences were found in the active tension developed with faster rate of stretch (duration of stretch 4.2 sec). In these conditions the average slope or gain constant in precollicular animals was 37 g/mm, with a maximum reflex tension of 337 g, in intercollicular animals 99 g/mm with maximum tension of 850 g.

Development of active tension usually started at the shortest extensions, but sometimes the threshold was higher so that there was no active tension until greater extensions were used. High thresholds were particularly common in precollicular animals (Fig. 3).

2. *Schiff-Sherrington release.* — The extensor tone of the forelimbs is controlled by two kinds of ascending tonic inhibition, viz: *a*) Schiff-Sherrington inhibition which arises in the lumbo-sacral segments of the spinal cord (31, 30), possibly in the border cells of Cooper and Sherrington (7, cf. 35) and *b*) reflex inhibition, which arises from hindlimb receptors (38, 39, 40). Stella (38, 40) has suggested that these two types of inhibition are identical but there is strong evidence that a considerable portion of the ascending inhibitory influence from the lumbo-sacral segments of the spinal cord is not driven reflexly by the hindlimb receptors (31, 30, 35). The effects of the postbrachial section of the spinal cord on the stretch reflex of the extensor muscles of the forelimb has been previously studied by Ruch and Watts (31) and Ruch (30). However a direct comparison of their results with ours cannot be made, because the authors recorded the reflex contractions to stretch from the lateral and medial head of the triceps together.

Novocaine block followed by surgical transection of the spinal cord at Th XII confirmed the increase of the reflex contraction to stretch of the caput longum of triceps as produced by this operation. Fig. 2 shows the reflex contraction of the triceps in slow (*A*) and fast (*B*, *C*) stretch of the muscle, before (curves 1) and after (curves 2) postbrachial section of the spinal cord. The experiment was made on a cat decerebrated at the intercollicular level, which led to strong rigidity as well as correspondingly strong reflex contractions to stretch up to 14 mm. After the Schiff-Sherrington operation the reflex contraction increased and the slope of the curve rose so that the effective myotatic loop gain became greater (see legend of Fig. 2). There was also a shift of the curve to the left so that the muscle already at zero extension developed some tension. This shift signifies a lowering of reflex threshold. In this as well as in some other experiments the flattening of the end portion of the curve may be explained on the basis of the previous considerations (see page 95). It is not surprising that in fast stretch the gain is higher than with slow pull (cf. 17). With fast stretch of the muscle the rise in tension was approximately twice as large as with slow stretch.

Fig. 3 is from a cat decerebrated at the precollicular level, which showed fissurate myosis during the whole experiment. The animal had strong rigidity in the hindlimbs, but not in the forelimbs. In order to produce a reflex, stretches were used whose average duration was 3.7 sec. In the decerebrate animal the muscle did not develop any active tension before 12 mm extension and the gain constant was only 7 g/mm (curve 1). Thus the stretch reflex was practically absent. However, after postbrachial section of the spinal cord development of some active tension started immediately (curve 2). The points of the curve lie on two crossing straight lines,

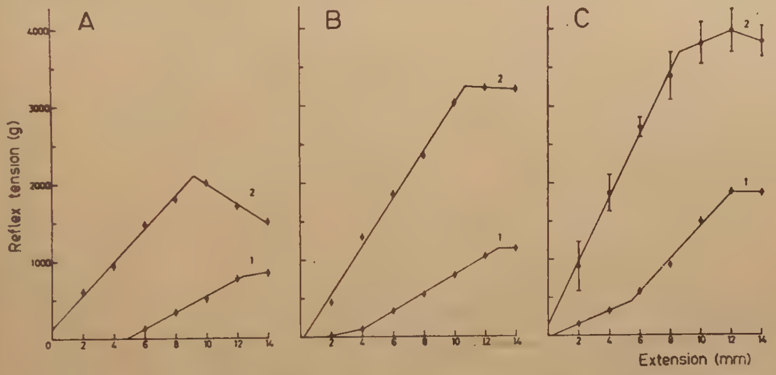

Fig. 2. – *Schiff-Sherrington phenomenon in intercollicular decerebrate cat.*

Plot of reflex contraction in stretch (g tension) against mm extension of caput longum of triceps before (curves 1) and 20 min after postbrachial transection of the spinal cord (curves 2). In this as well as in the following experiments the muscle was initially at zero extension. In *A*, *B*, *C* the average duration of the stretches is respectively 17.7, 3.6 and 1.0 sec. The slope or gain constants of the curves 1 are respectively 107, 118, 217 g/mm and those of the curves 2 are 217, 311, 415 g/mm. These values, here and elsewhere, have been derived from the straight lines drawn through the data. Uppermost curve in *C* refers to the average values of four stretches and the vertical lines are drawn on the basis of mean variations; lowermost curves in *B* and *C* refer to the average values of two stretches, while the other curves refer to single stretches.

the second of which has a gain constant of 52 g/mm. The experiment shows that the low degree of rigidity of the forelimbs as well as the low gain of the stretch reflex were due at least in part to a tonic inhibitory action from the caudal region of the spinal cord.

In the cases where the spinal transection was preceded by local novocainization, the release of the stretch reflex produced by the Schiff-Sherrington operation persisted without any sign of decrease for one and an half hour. During this time the reflex was periodically recorded.

It is concluded that postbrachial transection of the spinal cord produces a release of the stretch reflex of triceps characterized by some lowering of the threshold but mainly by an increase of gain. These changes in the tension-extension curve are present with slow stretch and emphasized by an increase in the rate of pull.

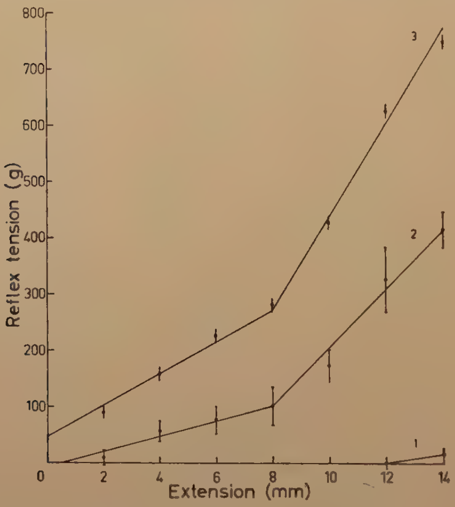

Fig. 3. – *Schiff-Sherrington operation followed by cerebellectomy in precollicular decerebrate animal.*

1: single stretch reflex in decerebrate preparation, showing that the muscle starts to develop some active tension at 12 mm extension (gain constant 7 g/mm); 2: 30 min after postbrachial section of the spinal cord (4 stretches); 3: 20 min after complete cerebellectomy made by suction. The gain constants of the uppermost part of curves 2 and 3 are respectively 52 and 67 g/mm. Average duration of stretch 3.7 sec.

3. *Cerebellar release.* — The strong rigidity which appears in otherwise intact as well as in decerebrate animals after complete cerebellectomy is related to the withdrawal of a tonic inhibitory influence, mainly exerted through the fastigial nuclei (cf. 2). The release phenomena which occur after cerebellectomy or bilateral destruction of the fastigial nuclei have been studied against the background of the stretch reflex in decerebrate animals. Fig. 4 shows the tonic stretch reflex of the triceps taken in a precollicular decerebrate animal before (curve 1) and after (curve 2) complete ce-

rebellectomy made by suction. While the points in curve 1 lie on one straight line with a myotatic gain constant of 9 g/mm, in curve 2 they fall on two straight lines, the first part of which shows a gain of 78 g/mm. In this experiment the threshold of the stretch reflex was unchanged. However in other cases the tension-extension curve started at a slightly higher level than in the decerebrate condition. The increased stretch reflex after cerebellectomy was also produced by making a bilateral electrolytic destruction of the fastigial nuclei in a number of animals. Increased rate of stretch augmented the gain constant. Thus in one experiment, not illustrated here, where the 14 mm stretch lasted 25.0, 5.0 and 1.0 sec respectively, the myotatic reflex in the decerebrate condition developed a tension of 160, 720 and 665 g respectively (gain constants 9, 83 and 80). This, after cerebellectomy, reached the values 740, 1030 and 1320 g respectively (gain constants 78, 86 and 112).

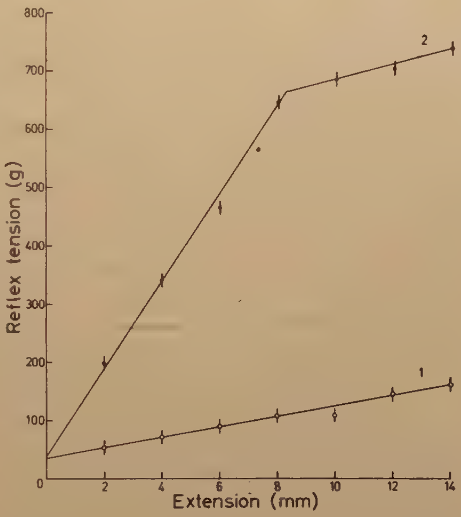

Fig. 4. – *Cerebellar release in precollicular decerebrate animal.*

Active tension developed during single stretch reflex before (curve 1) and 50 min after complete cerebellectomy made by suction (curve 2). The gain constant increased from 9 to 78 g/mm. Average duration of stretch 25 sec.

All records have been taken 20-60 min after cerebellar destruction. In none of these experiments was the increase of the stretch reflex due to cervical reflexes elicited by the opisthotonus, produced by cerebellectomy, because the head of the animal was rigidly fixed in the stereotactic instrument.

Summing up, complete cerebellectomy made by suction, as well as bilateral destruction of the fastigial nuclei, produced an increase of the tonic stretch reflex, manifesting itself mainly in the rise of the gain constant. The change in the slope of the curve was sometimes accompanied by slight lowering of the threshold of the reflex. These effects therefore were very similar to those produced by postbrachial transection of the spinal cord.

4. *Combined effects of Schiff-Sherrington and cerebellar release.* — The release of the stretch reflex produced by postbrachial transection of the spinal cord is further increased by complete cerebellectomy. Fig. 3 shows such an experiment. As was observed, the triceps muscle in this precollicular decerebrate animal started to develop some active tension at 12 mm extension and the gain was very low (7 g/mm). Curve 2 was taken 30 min after postbrachial section of the spinal cord, while curve 3 followed some time after cerebellectomy. The points of both curves 2 and 3 lie on two straight lines and the gain constants of the second part of the corresponding curves are respectively 52 and 67 g/mm. Clearly postbrachial section of the spinal cord as well as complete cerebellectomy both add to the stretch reflex of the triceps both doing it by augmenting the slope of the curve. The effect of cerebellectomy is far stronger on the slope than on the threshold of the reflex. These combined effects are seen not only in precollicular decerebrate animals, where the stretch reflex initially is strongly suppressed by active inhibition, but also in intercollicular animals, where the triceps usually develops a strong stretch reflex.

5. *Effects of unilateral destruction of the fastigial nucleus on the stretch reflex.* — Sprague and Chambers (36) found that a striking collapse of decerebrate rigidity occurred after total destruction of the ipsilateral fastigial nucleus. It has been shown by Moruzzi and Pompeiano (25) that the atonia which follows ipsilateral destruction of the fastigial nucleus is due to the fact that the postural mechanisms of the atonic side are easily suppressed by the inhibitory barrage arising in the spastic side of the body as soon as they are deprived of the support of the cerebellar facilitation. On the other hand the increase of rigidity on the spastic side of the body is due, at least in part, to some release produced by reduction of the crossed inhibitory barrage arising from the atonic side. If the contralateral fastigial nucleus is destroyed, the normal symmetrical fastigial control is eliminated but the chain of reflex action will go on, this

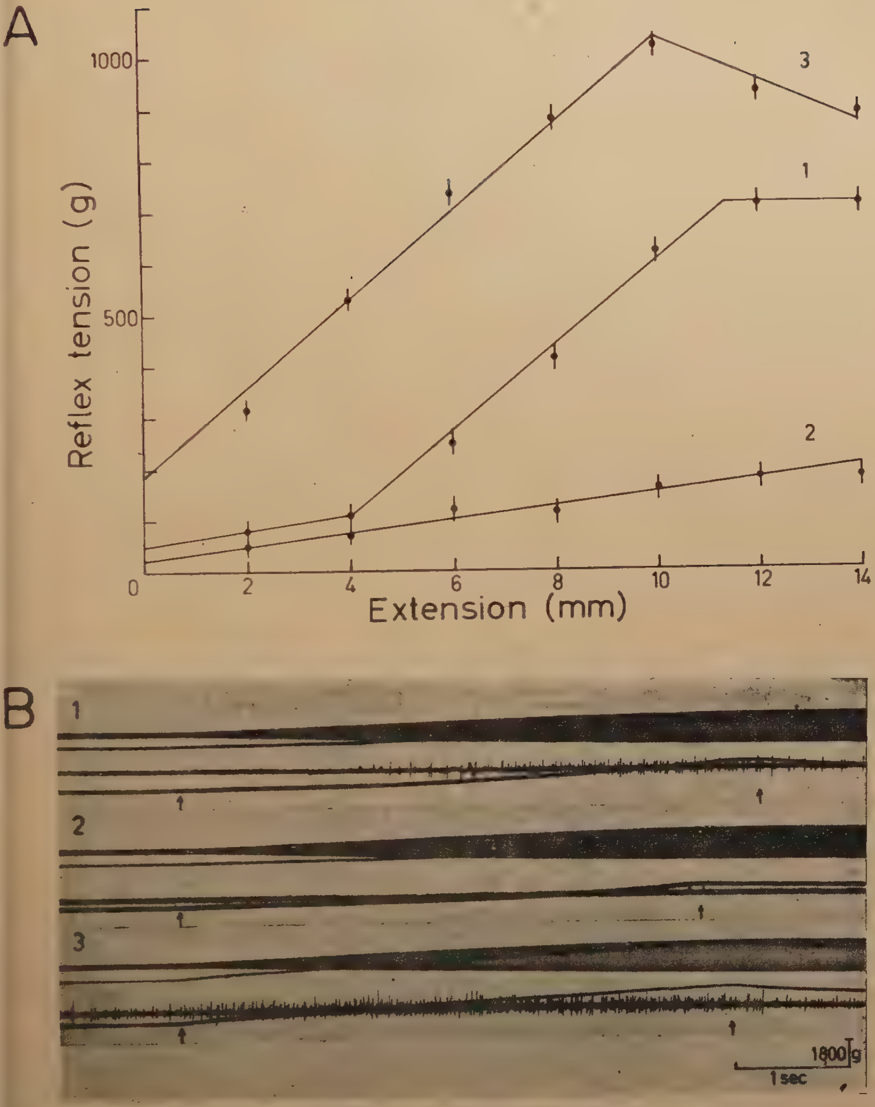

Fig. 5. – *Effects of electrolytic destruction of the fastigial nuclei.*

1: single stretch reflex in the intercollicular preparation; 2: 15 min after total destruction of the ipsilateral fastigial nucleus; 3: average values of 2 stretches taken 50 min after total destruction of the contralateral fastigial nucleus. The gain constants are 83, 13, 86 g/mm respectively. Average duration of stretch 5 sec. *B*: *Electromyograph records from the same experiment*. In each record the upper pair shows extension of the muscle and myogram, the lower electromyogram and myogram. The arrows indicate the duration of 14 mm stretch. The corresponding values of active tension have been plotted in *A*; similarly marked.

time in opposite direction, until a new equilibrium of ipsilateral extensor rigidity is achieved.

Fig. 5 *A* shows the effects of electrolytic destruction of the fastigial nuclei on the tonic stretch reflex. In this case the initial reflex tension in the decerebrate animal was quite high (gain constant 83 g/mm in curve 1). Then the fastigial nucleus of the ipsilateral side was completely destroyed (curve 2). The effectiveness of this lesion was shown by a hypotonia of the ipsilateral hindlimb as well as by an increase of rigidity in the contralateral limbs. The reduction of the stretch reflex was great and gain went down to only 13 g/mm.

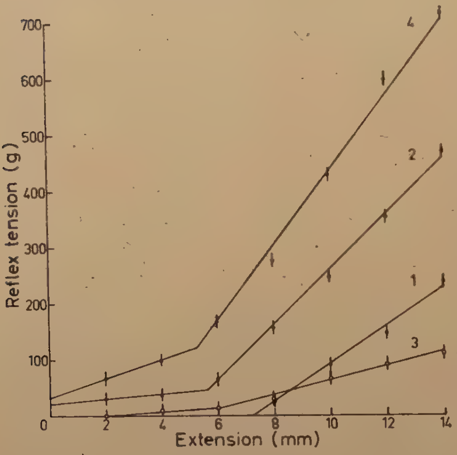

Fig. 6. – *Schiff-Sherrington operation followed by electrolytic destructions of the fastigial nuclei in precollicular decerebrate animal.*

1: stretch reflex in decerebrate preparation; 2: 30 min afte postbrachial transection of the spinal cord; 3: 15 min after tota destruction of the ipsilateral fastigial nucleus; 4: 20 min afte total destruction of the contralateral fastigial nucleus (2 stretches) The gain constants from 1 to are 33, 48, 12 and 66 g/mm respectively. Average duration o stretch 11.4 sec.

At the end of the experiment histological control confirmed the sit of the lesion. Then followed total destruction of the contralatera fastigial nucleus (curve 3), leading to strong symmetrical rigidity i hindlimbs. The gain constant rose to 86 g/mm.

It is of interest to observe that in this experiment the bilatera destruction of the fastigial nuclei did not change the slope of th tension-extension curve beyond the value seen in the original decerebrate condition (curve 1). However the curve shifted to the lef thus showing some reduction of threshold.

A reduction of the triceps stretch reflex after destruction of th ipsilateral fastigial nucleus can be observed also in decerebrat animals previously submitted to postbrachial section of the spin cord. Fig. 6 shows that the tension-extension curve in the decerebrate animal had a gain constant of 33 g/mm (curve 1), which increased after postbrachial section of the spinal cord to 48 g/m (curve 2). Now destruction of the ipsilateral fastigial nucleus (curve 3) produced a loss of gain to 12 g/mm which is less than in the decerebrate animal, in spite of the fact that the tension-extension curve started at a higher level than in the decerebrate condition. However, electrolytic destruction of the contralateral fastigial nucleus thereafter increased the slope of the curve to 66 g/mm in curve 4, which is more than was caused by the Schiff-Sherrington operation.

In a second group of experiments the decerebrate animal was submitted to total electrolytic destruction of the contralateral fastigial nucleus, which produced atonia of the limbs of the corresponding side. Fig. 7 shows the results of one experiment, made on a precollicular animal (curve 1). The active tension produced during the stretch reflex increased after destruction of the contralateral fastigial nucleus (curve 2), the gain constant now being 80 g/mm as against 16 g/mm in the decerebrate state.

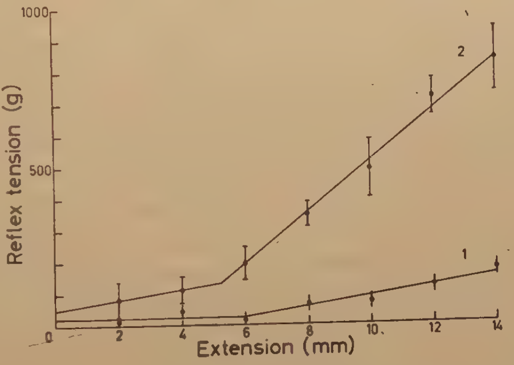

Fig. 7. – *Effects of electrolytic destruction of the contralateral fastigial nucleus in precollicular decerebrate animal.*

1: decerebrate control; 2: 30 min after total destruction of the contralateral fastigial nucleus (3 stretches). The gain constants are 16 and 80 g/mm respectively. Average duration of stretch 5.3 sec.

It is concluded that the total destruction of the fastigial nucleus of the contralateral side leads to changes of the triceps stretch reflex similar to those produced by bilateral and total destruction of the fastigial nuclei. However, as mentioned before, the release of the stretch reflex following destruction of the contralateral fastigial nucleus is probably due to reduction of the crossed inhibitory barrage arising in the atonic limbs, deprived of cerebellar facilitation. The peripheral mechanisms underlying the changes of the stretch reflex after unilateral fastigial lesion will be studied in the next section.

6. *Effects of contralateral deafferentation.* — Fig. 8 (curve 1, control 1) shows that after this operation (curve 2) both threshold

and slope of the curve have changed. The whole tension-extension curve shifted to the left and the gain constant reached 73 g/mm which is much higher than in the decerebrate state (39 g/mm). The effects of contralateral deafferentation on the triceps stretch reflex were present also in animals previously submitted to postbrachial transection of the spinal cord or complete cerebellectomy. Despite the remarkable release of the stretch reflex produced by the last operations, the deafferentation of the contralateral forelimb induced a further increase of the active tension developed by the muscle in response to the same stretch. In the last condition the gain constant after contralateral deafferentation was higher than in the normal decerebrate animal, probably because excitation was not concealed by the Schiff-Sherrington and cerebellar inhibitions.

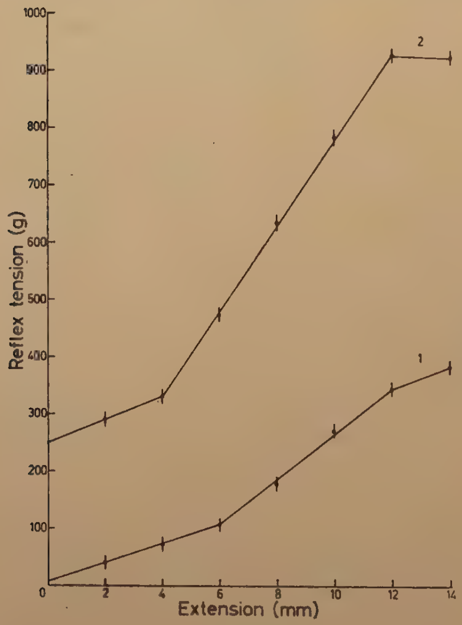

Fig. 8. – *Effects of deafferentation of the contralateral forelimb.*

1: precollicular decerebrate animal (2 stretches); 2: 20 min after deafferentation of the contralateral forelimb from C VI to Th I. The gain constant increased from 30 to 73 g/mm. Average duration of stretch 11.2 sec.

In Fig. 9 the slope of curve 1 in the decerebrate preparation was 38 g/mm and increased to 70 g/mm after postbrachial transection of the spinal cord (curve 2). It became 140 g/mm (curve 3) after deafferentation of the contralateral forelimb. However, the threshold was not significantly altered. Corresponding changes of the stretch reflexes were produced also by deafferentation of the contralateral forelimb made after cerebellectomy, all greater in fast stretch.

The increase of the triceps stretch reflex after deafferentation of the contralateral forelimb was undoubtedly due to release from tonic inhibition arising in the contralateral spastic leg. The alternative explanation, namely that crossed extension of the forelimb was caused by an injury discharge from the severed dorsal roots of the contralateral side could safely be dismissed, since: *a*) as a precaution 20 min were allowed after deafferentation before records were taken, and the increase of the stretch reflex remained unmodified during the rest of the experiment (up to 1 hour); *b*) the effect was present after novocainization of the central ends of the severed dorsal roots; *c*) the discharge of single motor units isolated in the muscle was regular and of low frequency.

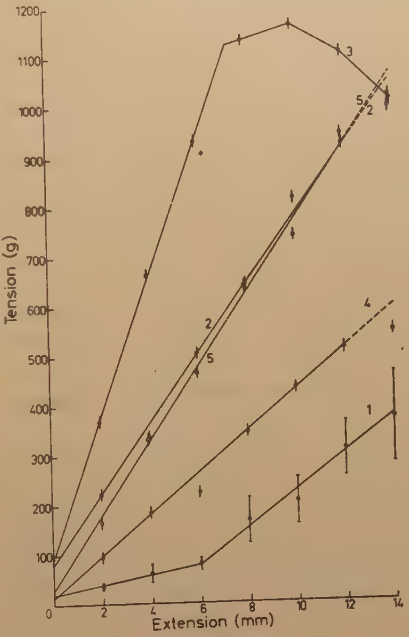

Fig. 9. – *Schiff-Sherrington operation, followed by deafferentation of forelimbs and cerebellectomy.*

1: average values of the active tension developed during three stretches in the precollicular decerebrate animal, gain constant 38 g/mm; 2: 30 min after postbrachial section of the spinal cord (two stretches), gain constant 70 g/mm; 3: 20 min after deafferentation of the contralateral forelimb from C VI to Th II, gain constant 140 g/mm; 4: 20 min after deafferentation of the ipsilateral forelimb from C VI to Th II, gain constant 42 g/mm; 5: 40 min after complete cerebellectomy made by suction (two stretches), gain constant 75 g/mm. Average duration of stretch 13.1 sec.

7. *Effects of the ipsilateral deafferentation.* — It was shown by Sherrington (32) and Liddell and Sherrington (17) that postural reflex contractions are greatly dependent on the proprioceptive inflow: in fact deafferentation produces flaccidity of the rigid limb in the decerebrate animal. In order to elucidate the role of the proprioceptive input in the release phenomena, produced by the

Schiff-Sherrington operation or by complete cerebellectomy, one might consider studying the effect of ipsilateral deafferentation on the stretch reflex as originally released by these two operations. However, such an experiment cannot solve the problem because the postural mechanisms of the deafferented forelimb are likely to be overruled by the inhibitory barrage from the contralateral forelimb as soon as the ipsilateral side is deprived of the support of proprioceptive facilitation. For these reasons the deafferentation of the ipsilateral forelimb was made on animals in which postbrachial section of the spinal cord or cerebellectomy were *preceded* by deafferentation of the contralateral forelimb.

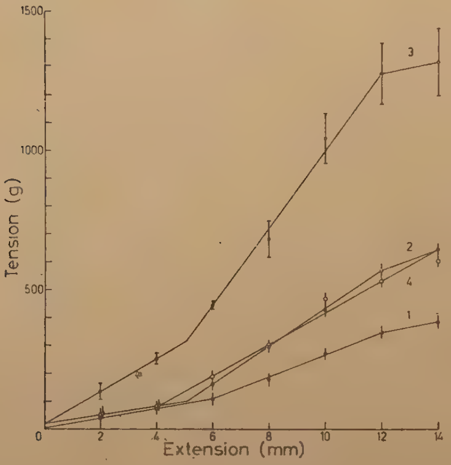

Fig. 10. – *Effects of ipsilateral deafferentation on the release produced by contralateral deafferentation followed by postbrachial section of the spinal cord.*

1: average active tension developed during two stretches in the precollicular decerebrate animal, gain constant 39 g/mm; 2: 30 min after deafferentation of the contralateral forelimb from C VI to Th II, gain constant 67 g/mm; 3: 20 min after postbrachial section of the spinal cord (four stretches), gain constant 138 g/mm; 4: 20 min after deafferentation of the ipsilateral forelimb from C VI to Th II, gain constant 57 g/mm. Average duration of stretch 11.9 sec.

Fig. 10 shows such an experiment. The slope of the linear portion of the tension-extension curve in this precollicular decerebrate cat gave a gain constant of 39 g/mm (curve 1) which reached 67 g/mm after deafferentation of the contralateral forelimb (curve 2). Transection of the spinal cord at Th XII added a remarkable increase to the gain constant (138 g/mm in curve 3), which fell to 57 g/mm (curve 4) after ipsilateral deafferentation. No change in threshold occurred in this experiment. The same results have been obtained also when, instead of postbrachial transection of the spinal cord, cerebellectomy was performed, thus suggesting that part of the release of the stretch reflex from Schiff-Sherrington and from cerebellar inhibition is tied to the integrity of the afferent input. Curve 4 of Fig. 10 does not contain any stretch reflex whatever but is simply a consequence of the constant discharge from α-motoneurones which leads to greater tension when the muscle is extended. Its slope shows what strong α facilitation can do by itself, owing to the mechanical properties of a muscle excited by a constant high output from its motoneurones.

The relatively pure effect of cerebellectomy or postbrachial section of the spinal cord on α activity was studied in animals previously submitted to bilateral deafferentation. In Fig. 9 curve 4 was obtained after postbrachial section of the spinal cord followed by bilateral deafferentation of the forelimbs. In this condition the muscle developed a tension slightly higher than in the original decerebrate state (gain constant 42 g/mm). When next complete cerebellectomy by suction (curve 5) followed, the slope of the tension-extension curve changed to 75 g/mm, thereby reproducing the curve (2) obtained in the normal decerebrate animal after the Schiff-Sherrington operation. Throughout the experiment of Fig. 9 threshold changes were negligible and slope changes dominant.

In another experiment on an animal with bilateral deafferentation following postbrachial section of the spinal cord, cerebellectomy by electrolytic destruction of both fastigial nuclei set up tension to a level higher than the one produced by the Schiff-Sherrington operation, so that the slope of the curve approached that obtained by contralateral deafferentation. Similar results were seen in other decerebrate animals in which bilateral deafferentation of the forelimbs was preceded by complete cerebellectomy followed by postbrachial transection of the spinal cord. These effects were present with both slow and fast stretch of the muscle.

8. *Release of the stretch reflex in spinal animals.* — It is well known that the extensor reflexes are depressed by spinal section (33). For example the knee-jerk becomes more susceptible to inhibition (1) and the stretch reflex disappears. The disappearance of the stretch reflex in the acute spinal animal means that the α-motoneurones do not respond to stretch of the muscle, when deprived of the facilitatory action of the brain stem centres. In some spinal animals we have confirmed these observations. In Fig. 11 the abscissa itself (= zero ordinates) must be taken to be tension-extension curve of triceps, because no active tension was developed during stretch. After postbrachial section of the spinal cord, a stretch reflex appeared and the gain constant of the linear portion of the curve (2)

became 37 g/mm. It is of interest that there was no active tension at short extensions, *i. e.*, the threshold of the stretch reflex was quite high. After deafferentation of the contralateral forelimb the gain of the curve (3) increased to 65 g/mm. This experiment shows: *a*) that in the spinal animal the stretch reflex of the triceps was suppressed by tonic inhibition arising both from the contralateral forelimb as well as from the lumbo-sacral segments of the spinal cord, and *b*) that consequently these inhibitory mechanisms are spinal in origin or have spinal components.

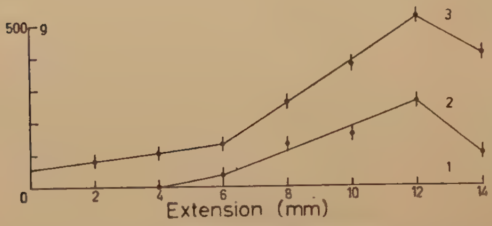

Fig. 11. – *Acute spinal animal with section of the spinal cord between C I and C II.*

Plot of the reflex contraction in g against extension. 1: shows that no active tension developed in the spinal state; 2: stretch reflex 10 min after postbrachial section of the spinal cord, gain constant now 37 g/mm; 3: 20 min after deafferentation of the contralateral forelimb from C VI to Th I (two stretches), gain constant 65 g/mm. Average duration of stretch 12.2 sec.

9. *Electromyographic records.* — Postbrachial section of the spinal cord, cerebellectomy and deafferentation of the contralateral forelimb are sometimes accompanied by a tonic contraction already at zero extension (Fig. 5 *A*). When in these conditions the electromyogram was recorded it never failed to show signs of increased activity, as compared against the background of the decerebrate state and same gain of the amplifier (Fig. 5 *B*). This increase in muscular activity was present in both normal and deafferented forelimbs, thus demonstrating that it did not necessarily require any afferent support.

It is often seen that during the stretch reflex there is recruitment of fresh neurones (12, 8, 14, 21). In our experiments the release of the tonic stretch reflex in animals with normal dorsal roots was regularly accompanied by recruitment of fresh units, more obviously so than in the decerebrate state used as control (Fig. 5 *B*).

It would be of interest to study the behaviour of single motor units during the different release phenomena at zero extension as well as during stretch. Occasionally such units were obtained. Thus in one case a unit that was silent at zero extension in the precollicular decerebrate state as well as after electrolytic destruction of the ipsilateral fastigial nucleus, fired at a rate of 10/sec after complete cerebellectomy. Its frequency further increased to 13-16/sec, after deafferentation of the contralateral forelimb. However, after ipsilateral deafferentation, the frequency of discharge at zero extension dropped to 6/sec. This fact is of interest because the single unit did not discharge in the precollicular decerebrate state. There is no reason to believe that in the latter condition the electromyogram failed to record from distant muscle fibres, because the muscle was explored over a large area.

With reference to the frequency changes of single motor units during stretch, the results illustrated by Fig. 12 give some useful information. These are from the experiment of Fig. 10. In this case electrical activity from single motor units could not be obtained, neither in the decerebrate state (precollicular) nor after deafferentation of the contralateral forelimb, despite the fact that a large muscular region was explored. Very little active tension developed in this preparation, so that the number of the active motor units (probably localized in the deepest part of the muscle) was quite small. There was probably strong inhibition from various sources. The release, produced by postbrachial transection of the spinal cord, however, brought in a unit whose spontaneous frequency of discharge at zero extension was around 6/sec and not significantly modified during stretch. This motor unit ceased to discharge after ipsilateral deafferentation. However, in other experiments some increase of

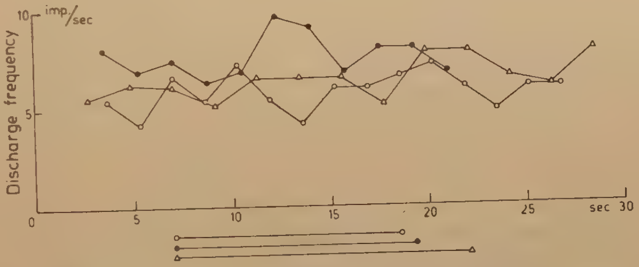

Fig. 12. — *Discharge frequency of a single motor unit of triceps during three stretches of 14 mm taken after Schiff-Sherrington operation.*

The respective stretch periods (11.5, 12.3, 15.1 sec) are similarly marked and placed below the diagram. The values of the active tension developed during the corresponding stretches will be found averaged in curve 3 of Fig. 10 (same experiment). The motor unit did not fire in the decerebrate state, nor after deafferentation of the contralateral forelimb, but did so after postbrachial section of the spinal cord. No significant change in frequency occurred during the stretches.

the frequency of discharge of single motor units within narrow limits could be observed during the slow stretch of the muscle.

All these changes refer to the tonic stretch reflex. In fast stretch single motor units are characterized by a slight increase of frequency during stretch. This increase of frequency is more marked after cerebellectomy or postbrachial section of the spinal cord than in the decerebrate animal. Of course no changes in frequency ever occurred after deafferentation of the corresponding forelimb.

The only information obtained from the electromyogram when the stretch reflex is reduced by destruction of the ipsilateral fastigial nucleus, is that abolition of electromyographic activity at zero extension is almost complete and that recruitment during stretch (Fig. 5) is reduced.

10. *Motor nerve stimulation.* — It appears from the study of Granit (14) made on the nerve-muscle preparation of soleus that if, in imitation of the reflex output, a slow rate of 14 shocks/sec be used, tension is proportional to extension, demonstrating that theoretically myotatic loop gain could be constant without recruitment of fresh neurones, merely on the basis of a constant efferent output. This finding has here been confirmed on the nerve-muscle preparation of triceps. At the same time it was proved, in agreement with the findings of Granit (14), that high stimulus frequencies (Fig. 13) and

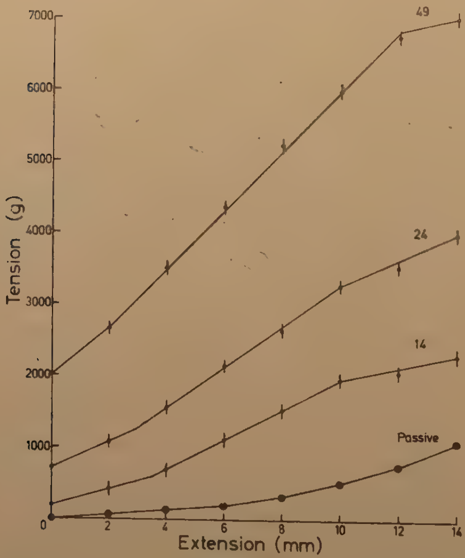

Fig. 13. – *Nerve-muscle preparation of caput longum of triceps.*

Tension-extension diagrams. Lowermost curve illustrates passive tension to stretch. The other curves show the maximum active tension produced at different extension by stimulating the radial nerve at the frequencies marked against the curves. Maximal stimulus strength was used. Duration of the tentani 1 sec. The gain constants are 210, 280, 410 g/mm at frequencies 14, 24 and 49 sec respectively.

increased stimulus strengths change the threshold as well as the slope of the curves. In the present experiments it has been found that during release of the stretch reflex, the spontaneous frequency of discharge of single motor units at zero extension may rise to a slightly higher level of frequency. In this way, even if one does not consider the recruitment of fresh units, and assumes that no change in frequency occurs during stretch, the observed increase of frequency of the tonic motor discharge, produced after " release ", should augment the gain constant and lower the threshold at which active tension in stretch becomes discernible.

DISCUSSION

The varieties of release occurring after postbrachial section of the spinal cord, cerebellectomy and deafferentation of the contralateral forelimb, studied in this paper, are likely to be dominantly of α type, because all of them have been observed in deafferented animals. There is evidence in fact that in the decerebrate cat the flaccidity of the bilaterally deafferented forelimbs (32) is replaced by a clearcut extensor rigidity following acute postbrachial transection of the spinal cord (18, 19, 38, 39, 34, 41, 42, 2) or following cerebellar ablation or anterior lobe topectomy (38, 37, 6, 34, 41, 42, 15) or bilateral electrolytic destruction of the fastigial nuclei (2). The extensor rigidity present in anaemically decerebrate cats despite complete deafferentation (28, 29) is explained by the fact that the anterior lobe has been infarcted by the anaemic procedure (38, 39, 40). Furthermore, the observations of Cardin (4) indicate that the crossed inhibition arising from the limb of one side affects also the deafferented limb of the contralateral side, suggesting that the α motoneurones of the extensor muscle of one side collapse under the impact of crossed inhibition.

In the analysis of the states of release the concept of loop gain has been employed. It was introduced by Granit (14) as a reference point in his work on the static relation between tension and extension in the soleus stretch reflex of the decerebrate preparation because this relation commonly was one of direct proportionality. Since both he and Denny-Brown (8) had found the output of each individual neurone to maintain its frequency in static stretch (cf. above), an increased gain would mean increased recruitment of fresh neurones during stretch, as known to occur (8, 14, 21) and confirmed above.

Furthermore, Matthews (21) had found that removal of gamma bias by cocainization of the small γ efferents (22, 23, 24, 20) only shifted the curve to the right in the tension-extension diagram, *i. e.*, did not remove gain but raised the threshold. Granit (14), furthermore, took up the question of whether, owing to purely muscular properties, the relation between tension and extension might not be linear also in the absence of recruitment, *i. e.*, with constant output, and proved that, when the nerve-muscle preparation of soleus was tetanized at constant slow rates, tension actually was proportional to extension, the slope of each curve depending upon stimulus strength and frequency employed. He therefore suggested that constant output was a degree of freedom of which the organism could avail itself on an α basis, meaning that a constant number of motoneurones were kept discharging at a constant rate. Some of his results were held to confirm this view. This mechanism of control would be a pseudo-stretch reflex since under such circumstances the afferent input would play a negligible role.

The first task was to investigate by the application of strong α release (see Figs. 9 and 10) to what an extent the "naturally" constant efferent output of the de-afferented animal would simulate the true reflex. It did so very well, and in particular in Fig. 9, the pseudo-reflex gave a perfect straight line for the relation between tension and extension with a gain constant as high as 75 g/mm. As in Granit's work the result could be imitated by tetanizing the motor nerve (Fig. 13). It is true, therefore, that a constant physiological output, as distinct from the constant artificial output of Fig. 13, is an available degree of freedom for regulating the relation between tension and extension according to Hooke's law. With a constant output the gain in stretch, indeed, is constant and depends upon a fixed neuronal setting, leding to a fixed discharge of the α-motoneurones. Rather remarkable, and hitherto not fully understood, is that with a variable input, such as in stretch with intact dorsal roots, linearity of the curves often is well maintained, despite obvious recruitment, as now again seen in the present work. Often, however, as pointed out by Granit (14) and Matthews (21) and verified above, the curve is better described by two linear portions and it may well be that the second one in such cases signifies recruitment (14). If the afferent input were of little significance relative to a decisive component of α release, a linear relation between tension and extension would be expected as in Fig. 9, but this and Fig. 10

(cf. curves 3 and 4) show quite clearly that de-afferentation actually does away with a considerable component of purely reflex gain which has to be compensated for by adding further α release through one more of the operations available for this purpose.

A characteristic feature of α release and one in which it differs from the γ release, so dominant in the soleus of the intercollicular cat (see e. g. 14, 22, 23, 24, 21), has been shown to be its strong influence on the magnitude of the gain constant. The effects of the operations employed have been on the slope of the curves rather than on their position in the tension-extension diagram. It is clear from the experiments with pseudo-reflexes and their imitation in the muscle-nerve preparation that this feature of α release is inevitable. The greater the number of active α neurones, the greater in those experiments was the slope (or its equivalent, the gain constant). Similarly, of course, if the number of actively discharging neurones undergoes a significant increase, also the threshold must diminish somewhat (the curve shift to the left) because the muscle will then be in a contracted state already at zero extension, as in Fig. 5. If, as e. g. in Fig. 4, the slope increases without marked effects on the threshold (without marked shift to the left of the curve), the interpretation is that the major part of the "release" has been spent in raising a large number of motoneurones from the realm of the subliminal fringe to a higher level of subthreshold excitability. This sensitive fringe creates a particularly favourable situation for the myotatic afferent input whose effect then emerges as good recruitment with a high gain constant. Thus in Figs. 9 and 10, threshold effects were small but effects on the slope of a large order. De-afferentation led to a considerable loss of gain showing that the afferents had activated a subliminal fringe, previously sensitized by the operations that had been performed in order to release α neurones. It is legitimate to predict that whenever the myotatic input plays on a sensitized subliminal fringe, the reflex will be sensitive to γ control of the spindles. Otherwise the results of Matthews and Rushworth (22, 23, 24), according to which the stretch reflex disappears or greatly diminishes after cocainization of the γ efferents, would be difficult to understand.

Considering that all varieties of α release differ from γ release in their strong effect on the gain constant it was pertinent to ask whether the setting of the level of α excitability might not be determined by a common factor, which would be the state of balance of tonic excitation against tonic inhibition in a common spinal centre of integration. Since the release mechanisms are polysynaptic, this centre would be located to premotor spinal interneurones. To investigate this possibility the two available mechanisms in the spinal animal, the Schiff-Sherrington release and the similar effect of severing the contralateral dorsal roots, were tried and actually found to produce a reflex with a gain constant of 65 g/mm (Fig. 11) in marked contrast to the original zero gain before these operations. This result shows *a*) that the well-known suppression of tonic stretch reflexes of extensors in the acute spinal animal (17, 1, 10) is due not only to lack of supraspinal excitatory support but also is a consequence of tonic inhibition from the two sources removed by the operations used (and possibly others not specifically tested) and *b*) that, in accordance with the assumption made, there is a spinal internuncial centre fully capable of mobilizing the extensor motoneurones to the point necessary for raising a good stretch reflex. There is therefore nothing to exclude the hypothesis that this spinal centre is the final integrator for forelimb extensors in the sense that its "setting" is a final evaluation of the various excitatory and inhibitory forces from spinal and supraspinal sources. This hypothesis implies that gain largely is determined by this balanced "evaluation", inasmuch as gain is neuronal and not muscular.

The importance of α setting of the gain of tonic stretch reflexes emerges with great clarity from the present study. This may partly be due to the selection of conditions calculated to emphasize this particular aspect. As has been pointed out above, conditions may also be selected so as to emphasize the setting of γ control (see e. g., 13, 22, 23, 24, 21). One might argue that the methods here employed (effectively cerebellectomy, section of the spinal cord below the entrance of the brachial plexus or of the contralateral dorsal roots) have been highly pathological. While this is true, the criticism, however, is hardly to the point. The operations have served to reveal the existence of powerful excitatory and inhibitory tonic influences, well known from previous work quoted, but here studied from the point of view of muscle control. Since these influences are so powerful, they can hardly be negligible and must be held in check. They represent equally many ways for the normal animal of dealing with motor situations by graded release according to the needs of the moment. Perhaps, since such mechanisms of control often would have to be released on the strength of information from the muscle sense organs, the creation of a sensitive subliminal fringe for the myotatic afferents to play upon is the most likely way of making them useful in the life of the organism.

SUMMARY

1. The effects of the release phenomena on the stretch reflex of the cat's forelimb triceps muscle has been studied. The relation between active tension and extension was observed when stretch was applied at both slow and fast constant rates. This relation tended to be linear. The slope was used to judge reflex gain across the spinal cord (the gain constant).

2. Postbrachial section of the spinal cord, deafferentation of the contralateral forelimb and complete cebellectomy or bilateral destruction of the fastigial nuclei, made on a background of Sherringtonian rigidity, produced an increase of the tonic stretch reflex, mainly due to a rise of the gain constant. These changes in the slope of the curve were sometimes accompanied by slight lowering of the threshold of the reflex so that the muscle already at zero extension developed some tension. All the effects on the tension-extension curve were present with slow stretch and emphasized by increasing the rate of pull.

3. All varieties of release employed added to the stretch reflex of the triceps by augmenting gain as judged by the slope of the curve, not only in initially precollicular animals, whose stretch reflexes are strongly reduced by active inhibition, but also in intercollicular ones, with their strong basic stretch reflexes.

4. The effects of postbrachial section of the spinal cord and of cerebellectomy on the tension-extension curves of triceps, obtained in otherwise normal decerebrate animal, could also be obtained against a background of pure α rigidity, *i. e.*, after all myotatic influences had been abolished by acute bilateral deafferentation. In this condition the increase of active tension which occurs during stretch is not reflex at all but simply a consequence of the constant output grom the α-motoneurones (pseudo-stretch reflex) in combination with mechanical properties of muscle. Also in pseudo-stretch reflexes active tension was linearly related to extension.

5. However, the release of the stretch reflex from cerebellar and spinal cord ascending inhibition is tied at least in part to the integrity of the afferent input. In fact deafferentation of the ipsila-

teral forelimb in animals, previously submitted to postbrachial section of the spinal cord or complete cerebellectomy, produced a loss of gain.

6. In the acutely spinal animal the stretch reflex of the triceps was absent but could be obtained after postbrachial section of the spinal cord or contralateral deafferentation, thus demonstrating not only that the release from such tonic inhibition is spinal in origin or has a spinal component but also that a spinal premotor centre in itself can raise rigidity and tonic stretch reflexes.

REFERENCES

1. Ballif, L., Fulton, J. F. and Liddell, E. G. T. Observations on spinal and decerebrate knee-jerks with special reference to their inhibition by single break-shocks. *Proc. Roy. Soc., B, 98*: 589-607, 1925.
2. Batini, C., Moruzzi, G. and Pompeiano O. Cerebellar release phenomena. *Arch. ital., Biol., 95*: 71-95, 1957.
3. Cardin, A. Rigidità da decerebrazione negli arti posteriori deafferentati. *Boll. Soc. ital. Biol. sper., 22*: 81-83, 1946.
4. Cardin, A. Sul meccanismo della inibizione reciproca degli arti posteriori dell'animale decerebrato. *Boll. Soc. ital. Biol. sper., 22*: 607-608, 1946.
5. Cardin A. Influenza della deafferentazione di uno o più arti sul tono posturale degli arti, nell'animale normale. *Boll. Soc. ital. Biol. sper., 22*: 609-611, 1946.
6. Chambers, W. W. and Sprague, J. M. Role of the cerebellum in regulation of decerebrate spasticity in the cat. *Fed. Proc., 11*: 23-24, 1952.
7. Cooper, S. and Sherrington, C. S. Gower's tract and spinal border cells. *Brain, 63*: 123-124, 1940.
8. Denny-Brown, D. On the nature of postural reflexes. *Proc. Roy. Soc., B, 104*: 252-301, 1929.
9. Denny-Brown, D. and Liddell, E. G. T. Some observations on the reflex activity of a muscle of the forelimb (M. supraspinatus). *Quart. J. exp. Physiol., 16*: 353-371, 1927.
10. Denny-Brown, D. and Liddell, E. G. T. The stretch reflex as a spinal process. *J. Physiol., 63*: 144-150, 1927.
11. Eldred, E., Granit, R. and Merton, P. A. Supraspinal control of the muscle spindles and its significance. *J. Physiol., 122*: 498-523, 1953.
12. Fulton, J. F. and Liddell, E. G. T. Electrical responses of extensor muscles during postural (myotatic) contraction. *Proc. Roy. Soc., B, 98*: 577-589, 1925.
13. Granit, R. *Receptors and sensory perception.* New Haven, Yale Univ. Press, xii + 369 pp., 1955.
14. Granit, R. Neuromuscular interaction in postural tone of the cat's isometric soleus muscle. *J. Physiol., 143*: 387-402, 1958.
15. Granit, R., Holmgren, B. and Merton, P. A. The two routes for excitation of muscle and their subserviences to the cerebellum. *J. Physiol., 130*: 213-224, 1955.
16. Hunt, C. C. The reflex activity of mammalian small-nerve fibres. *J. Physiol., 115*: 456-469, 1951.
17. Liddell, E. G. T. and Sherrington, C. S. Reflexes in response to stretch (myotatic reflexes). *Proc. Roy. Soc., B, 96*: 212-242, 1924.
18. Liljestrand, G. und Magnus, R. Über die Wirkung des Novokains auf den normalen und den tetanusstaren Skelettmuskel und über die Entstehung der lokalen Muskelstarre beim Wundstarrkrampf. *Pflügers Arch., 176*: 168-208, 1919.
19. Magoun, H. W., Hare, W. K. and Ranson, S. W. Rôle of the cerebellum in postural contractions. *Arch. Neurol. Psychiat., Chicago, 37*: 1237-1250, 1937.

20. MATTHEWS, P. B. C. The effect of the local application of procaine on the stretch reflex of the soleus muscle of the cat decerebrated by anaemia. *J. Physiol.*, *140*: 408-420, 1958.

21. MATTHEWS, P. B. C. The effect of the activity of the y-motoneurones on the relation between tension and extension in the stretch reflex. *J. Physiol.*, *140*: 54-55 P, 1958.

22. MATTHEWS, P. B. C. and RUSHWORTH, G. The selective effect of procaine on the stretch reflex and tendon jerk of soleus muscle when applied to its nerve. *J. Physiol.*, *135*: 245-262, 1957.

23. MATTHEWS, P. B. C. and RUSHWORTH, G. The relative sensitivity of muscle nerve fibres to procaine. *J. Physiol.*, *135*: 263-269, 1957.

24. MATTHEWS, P. B. C. and RUSHWORTH, G. The discharge from muscle spindles as an indicator of efferent paralysis by procaine. *J. Physiol.*, *140*: 421-426, 1958.

25. MORUZZI, G. and POMPEIANO, O. Inhibitory mechanisms underlying the collapse of decerebrate rigidity after unilateral fastigial lesions. *J. comp. Neurol.*, *107*: 1-26, 1957.

26. PERL, E. R. Crossed reflex effects evoked by activity in myelinated afferent fibers of muscle. *J. Neurophysiol.*, *21*: 101-112, 1958.

27. PERL, E. R. Effects of muscle stretch on excitability of contralateral motoneurones. *J. Physiol.*, *145*: 193-203, 1959.

28. POLLOCK, L. J. and DAVIS, L. The reflex activities of a decerebrate animal. *J. comp. Neurol.*, *50*: 377-411, 1930.

29. POLLOCK, L. J. and DAVIS, L. Studies in decerebration. VI. The effect of deafferentation upon decerebrate rigidity. *Amer. J. Physiol.*, *98*: 47-49, 1931.

30. RUCH, T. C. Evidence of the non-segmental character of spinal reflexes from an analysis of the cephalad effects of spinal transection (Schiff-Sherrington phenomenon). *Amer. J. Physiol.*, *114*: 457-467, 1936.

31. RUCH, T. C. and WATTS, J. W. Reciprocal changes in reflex activity of the forelimbs induced by post-brachial " cold-block " of the spinal cord. *Amer. J. Physiol.*, *110*: 362-375, 1934.

32. SHERRINGTON, C. S. Decerebrate rigidity, and reflex coordination of movements. *J. Physiol.*, *22*: 319-322, 1898.

33. SHERRINGTON, C. S. Flexion-reflex of the limb, crossed extension-reflex and reflex stepping and standing. *J. Physiol.*, *40*: 28-121, 1910.

34. SPRAGUE, J. M. Role of spinal cord in regulation of decerebrate spasticity in the cat. *Fed. Proc.*, *11*: 152-153, 1952.

35. SPRAGUE, J. M. Spinal " border cells " and their role in postural mechanism (Schiff-Sherrington phenomenon). *J. Neurophysiol.*, *16*: 464-474, 1953.

36. SPRAGUE, J. M. and CHAMBERS, W. W. Regulation of posture in intact and decerebrate cat. I. Cerebellum, reticular formation, vestibular nuclei. *J. Neurophysiol.*, *16*: 451-463, 1953.

37. SPRAGUE, J. M., SCHREINER, L. H., LINDSLEY, D. B. and MAGOUN, H. W. Reticulo-spinal influences on stretch reflexes. *J. Neurophysiol.*, *11*: 501-507, 1948.

38. STELLA, G. Sul meccanismo della rigidità da decerebrazione in arti deafferentati. *Atti Soc. med. chir. Padova*, *23*: 5-16, 1944.

39. STELLA, G. Influenza del cervelletto sulla rigidità da decerebrazione. *Atti Soc.med. chir. Padova*, *23*: 17-21, 1944.

40. STELLA, G. Nuove osservazioni sull'attività reflessa tonica del cervelletto nell'animale decerebrato. *Boll. Soc. ital. Biol. sper.*, *22*: 78-80, 1946.

41. TERZUOLO, C. and TERZIAN, H. Cerebellar increase of postural tonus after deafferentation and labyrinthectomy. *J. Neurophysiol.*, *16*: 551-561, 1953.

42. TERZIAN, H. e TERZUOLO, C. Le componenti automatiche e riflesse del tono posturale. *Arch. Fisiol.*, *54*: 37-61, 1954.

Arch. ital. Biol., *98*: 92-117, 1960.

ANALYSES

H. S. Carslaw and J. C. Jaeger. *Conduction of heat in solids.* Oxford University Press, London, 1959, VIII-510 pp., 84 s.

This book is an important reference work for physiologists interested in the quantitative aspects of their subject. It is an enlarged revision of a first edition which has already become a classic and has been out of print for several years. Although the book is written mainly for physicists and engineers, its relevance to physiology is indicated by the discussion and examples given below.

The usefulness of this book is not limited to problems in heat conduction: it extends to many physically and mathematically analogous problems such as diffusion, nerve electrotonus, viscous motion, the flow of fluids through porous media and others noted (with numerous references) on pages 28-29 of this book. For example, Fick's law of diffusion (1855) was based upon Fourier's law of heat conduction (1822), and the same mathematical methods apply to both diffusion and heat conduction problems. Solute concentration corresponds to temperature, solute flux to heat flux, and diffusion coefficient to thermal conductivity. When a physiologist is willing to idealize and formalize a physiological diffusion problem to one with explicit boundary conditions defined for plane, cylindrical or spherical surfaces, (and with explicit initial conditions in the case of transients), then the chances are good that one of the many mathematical solutions collected in this book will be appropriate. Many of these cases have been restated for diffusion problems in *The Mathematics of Diffusion* by J. Crank, Oxford Press, 1956. Several cases relevant to synaptic physiology are illustrated by Eccles and Jaeger (*Proc. Roy. Soc. B, 148*: 38-56, 1957).

The mathematical treatment of passive membrane electrotonus along nerve axons dates back to the work of Hermann (1872), who recognized the analogy with heat conduction problems. The analogy with Kelvin's (1856) submarine telegraph cable was not recognized until later (Hoorweg, 1898, followed by Cremer and Hermann); this treatment is specifically analogous to the conduction of heat in a rod or wire. Heat loss by radiation from the rod into the surrounding medium corresponds to the " leakage " of current across the membrane in the case of electrotonus; temperature corresponds to electrotonic potential, heat capacity per unit length of rod corresponds to electric capacity per unit length of axon, and the longitudinal flux of heat in the rod corresponds to longitudinal current flow in the axon. Equation (7) on p. 135 of this book expresses a mathematical solution which corresponds to the transient spread of electrotonic potential in an axon of infinite length when the membrane potential at $x = O$ is suddenly displaced from its resting value by a constant amount; this equation (7) is transformed to conventional neurophysiological notation by setting $\nu = 1 / \tau$ and $k = \lambda_2 / \tau$ (where λ and τ are the usual length and time constants). A similar solution (differing only by one minus sign) corresponds to the application of a current step instead of a voltage step at $x = 0$ when $t = 0$; this solution is the same as that given by Hodgkin and Rushton (*Proc. Roy. Soc. B, 133*: 444, 1946, equation 4.1), and by Davis and Lorente de No (*Studies from the Rockefeller Institute, 131*: 1947, equation 37, p. 452).

Recent experimental developments in the use of intracellular microelectrodes and in the use of electronic voltage or current clamping circuits provide the possibility of experimentally realizing physical boundary conditions (in experiments with neurons *in situ*), which should correspond quite well to mathematical step boundary conditions. In problems involving soma-dendritic electrotonus (cf. Rall: *Science, 126*: 454, 1957), it is necessary to include soma-dendritic coupling in one of the mathematical boundary conditions.

For the benefit of mathematically inexperienced physiologists who wish to make use of solutions collected in this book, the importance of carefully considering the initial and boundary conditions (see section 1.9 of this book) should be emphasized. For those with more mathematical experience, who wish to derive solutions not listed in the book, the methods of reducing general problems to combinations of simpler problems (section 1.14) are very important; also, Chapter XII provides an introduction to the use of Laplace transforms which can produce rich rewards.

Another device deserving special mention is the instantaneous point source (sections 10.1 *et seq.*) which should be kept in mind as often providing a useful approximation to the spatio-temporal spread of heat (or substance, or charge) which is released or produced in a small region over a small interval of time. An example of instantaneous point charge in one dimension is provided on pp. 330 and 363 of Fatt and Katz (*J. Physiol., 115*: 1951), while an instantaneous point release of acetylcholine has been considered by Fatt (*Physiol. Rev., 34*: 1954, p. 704) and by Castillo and Katz (*J. Physiol., 128*: 1955.p.166). Chapter X presents several generalizations of this method to include movement, time variation and spatial distributions of sources and sinks.

W. Rall

G. Gamow. *Matter, Earth and Sky*. MacMillan and Co. Ltd., London, 1959, IX-593 pp., 50 s.

Professor Gamow is well known both for his original contributions to physics and for his ability to explain abstruse doctrines in a simplified form adequate for the general reader.

In the field of nuclear physics and cosmology his contributions include a theory on the escape of nuclear particles through the nuclear potential barrier (the so-called "tunnel effect" which Gamow proposed at the age of 24 to explain radioactive decay), a theory on the formation of heavier elements through neutron capture (developed with R. Alpher and H. Bethe, and appropriately called "alphabetic theory") and a theoretical reconstruction of the relative roles of radiant energy and of matter in the history of the universe. In 1954 Gamow (*Nature, 173*: 318, 1954) advanced a hypothesis (later elaborated by Crick, Griffith and Orgel, *Proc. Nat. Acad. Sci., 43*: 416-421, 1957) to explain how four nucleotides in a nucleic acid may determine the sequence of twenty amino acids in a protein molecule, by suggesting that twenty different triplets of nucleotides may have a specific affinity each for one amino acid.

Gamow's previous books include: The Creation of the Universe, The Birth and Death of the Sun, Biography of the Earth, One, Two, Three... Infinity, and the perhaps less popular but highly entertaining Mr. Tompkins in Wonderland, in which relativity is explained by describing the curious happenings which take place in a fantastic city in which the speed of light is only 20 miles per hour.

This last book on Matter, Earth and Sky covers briefly but systematically the most important topics of physics and might well be regarded as an unconventional textbook. As the author writes in the Preface, "The plan selected for this book is that of a trilogy dealing first with things our size, secondly with things much smaller, and finally with things much larger than ourselves" (p. VII). In accordance with this plan, the dimensions of material objects extending from 10^{-13} cm (the size of elementary particles) to 10^{26} cm (the size of clusters of galaxies) are displayed in the first illustration. Clearly, in order to cover such a wide field in one volume, a good deal of detail must be sacrificed but Gamow is very successful, not only in explaining in this short space the fundamental conclusions regarding each subject, but, in many cases, also in describing the evidence upon which such conclusions are based.

The section on classical physics (which Gamow considers important but unexciting) deals with some properties of solids, liquids and gases; the laws of mechanics (including mention of Eddington's criticism to Newton's first law); vibrations and waves (with a short section on speech and hearing); heat and "the mysterious entropy"; electricity; and light and radiations (with a section on vision). Description of the measurements of the velocity of light leads to discussion of Michelson's experiments, and a brief presentation of Einstein's special theory of relativity concludes the section on classical physics.

The second part on "things smaller than we are" describes first Brownian movements and Nernst's Heat Theorem, proceeding then rapidly to the physical properties of molecules, the kinetic theory of gases and the solid structure. One of the three subsequent chapters on chemistry deals with "The chemistry of life" and may well prove to be of interest to those biologists who have not kept up to date with their biochemistry.

The following chapters on atomic structure and properties describe J. J. Thomson's experiments on electrical conductivity of gases, the discovery of the electron, the determination of its charge to mass ratio and Thomson's theory of atomic structure. An account is then given of the experiments which led Rutherford to propose a different (planetary) model of the atom, and of those which led to the finding that the nuclear charge is proportional to the atomic number. The problems arising from attempts to reconcile the law of equipartition of energy with the observable behaviour of radiant energy in Jeans's reflecting cube or in a black body are then mentioned and it is explained how Plank's hypothesis on the quantal composition of radiant energy turned out "to save the world from the perils of Jeans's ultraviolet catastrophe" (p. 290). Bohr's atom and Pauli's exclusion principle are then mentioned and it is shown how the new theories on atomic structure provide a simple explanation of the periodic properties of elements, empirically described by Mendeleev. The last chapter on "small things" deals with nuclear physics, starting with radioactivity and ending with the properties of nuclear component particles and with nuclear fission.

The concluding section is concerned with the earth, the planets, the sun, the stars and cosmology. Gamow explains how the study of earthquakes has provided important information on the structure of the earth and describes how the geological history could be reconstructed from the study of sediments and fossils. A chapter in this section deals with meteorology. Present views on the origin of planetary systems are discussed and the methods used to determine distances, sizes and velocities of celestial bodies are mentioned. The final chapter on cosmology introduces some concepts of general relativity and discusses the expanding universe, the origin of chemical elements and present views on early history of the universe, ending with a brief mention of Bondi, Gold and Hoyle's proposal that matter is continuously created.

In this reviewer's opinion there is some confusion at pp. 136 and 137 in the arguments presented to explain why one's image in a flat mirror appears turned left to right rather than upside down. Some biologists will also disagree with the statement that "neurones of the brain are capable of only two states" (p. 131) just like the electronic tubes of computers, but they will be relieved by reading at the end of the book that "in spite of the often-used analogy between the brain and an electronic computer, we are still quite far from a physical explanation of mental processes" (p. 561).

Gamow's provocative way of writing, and the swift progress of his presentation from the classical to the very modern, are most effective in keeping the reader's interest constantly alive.

Although the book is " Dedicated to aspiring youth " it may well prove both enjoyable and useful to many adult readers.

M. G. F. FUORTES

J. A. V. BUTLER. *Inside the living cell.* George Allen and Unwin, London, 1959, 174 pp., 21 s.

Only the first part of this book, describing basic life processes such as synthesis of complex molecules, reproduction and energy exchange in cells, bacteria and viruses, deals with the subjects suggested by the title; the second part deals instead with properties of complex organisms and with some aspects of nerve function, while the last chapters include a discussion of such topics as instincts, memory, consciousness, free will, etc.

The link between these two parts is not altogether clear: the author appears to be more at his ease when he discusses the fundamental features of chemical organization of living structures, and many readers will find the first part of the book particularly valuable.

The initial chapters contain a brief description of protein structure and of the properties of enzymes and vitamins. An account is then given of recent views on protein synthesis and mention is made of the possible role of ribonucleic acid. Important recent conclusions on genetics and on viruses are then summarized and the embryological problem of cell differentiation is discussed as an introduction to a group of chapters on properties of organisms, which includes an account of hormones, antibodies and cancer.

A chapter on the origin of life and energy on exchange divides the part dedicated to general cellular biology from the second part on special functions. Discussion of origin of life is based on the assumption that " There must have been a time when the earth was too hot for any living things as we know them " (p. 100), while it is now considered more likely that the earth originated from accretion of relatively cold, solid particles (see, for instance, A. C. B. Lovell, *The individual and the universe*, Oxford University Press, 1958, pp. 35 and 36, or Gamow's book, reviewed above).

The following discussion on brain activities and on mental performances is also based to some extent on the debatable premise, that " The synapse.... gives an all-or-none response; if the signal is strong enough, it passes; below a certain strength nothing happens " (p. 143). In contrast with this assumption, there appears to be now enough evidence to justify the view that the transformation of all-or-none nerve impulses into graded activity is an important step in synaptic functions.

The last chapters on general properties of life and on complex mental performances are not very conclusive and some of the final remarks (for instance, that living things " exist in their own right as emergent creations of the 'elan vital' ".... and that " Mentality.... is implicit from the start, waiting to be realized ", p. 161) fail to convey a clear meaning. Still, it would be naive to expect that clear and simple answers could be given to the abstruse questions proposed in these final chapters and there is a value in simply stating what some of the important questions are.

Butler's book is certainly no substitute for the detailed information which one could gather by reading the original articles but should be sufficient to give to the non-specialist a broad view of the present knowledge in extensive fields of biology.

M. G. F. FUORTES

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AND COGNATE MEDICAL SCIENCES

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Vol. 1 *No. 1*

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Direttore responsabile: Prof. Giuseppe Moruzzi
Editore: F. Vallerini - Pisa
STABILIMENTI TIPOGRAFICI « E. ARIANI » E « L'ARTE DELLA STAMPA » - FIRENZE
Autorizzazione del Tribunale di Pisa N. 20/1956 del 1° Dicembre 1956